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ACTA PÆDIATRICA

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From the University Pediatric Hospital, Rikshospitalet, Oslo, Norway.
(Head: Professor L. SALOMONSEN, M. D.)

Creatinine Tolerance Test as a Renal Function Test

by ELI NORBYE

Creatinine is the anhydride of methylguanidine acetic acid (creatine), and is found normally in the organism together with creatine, mostly in the muscular tissue. Creatine, as also creatinine, is assumed to be formed in the muscles, creatine by synthesis of various amino acids. No agreement exists, however, as to how the creatinine is formed. Some investigators assume that it is not formed from creatine but, in addition to this, as a product of the protein metabolism (BEHRENDT). In the muscular tissue are found only relatively small quantities of creatinine as compared with creatine, while in blood plasma normally 80 per cent of the "total creatinine" is present as creatinine (BUCHT). It is held that under normal conditions creatinine is found in blood plasma in a concentration below 1.5 mg/100 ml, most frequently around 1 mg/100 ml.

Creatinine is excreted through the kidneys, through the glomeruli as well as the tubules, and the amount excreted diurnally is related directly to the muscular bulk. The excretion of creatinine is independent of protein intake. On ingestion of creatinine, however, the creatinine level of the blood rises simultaneously with elevation of excretion through the kidneys. This rise in blood creatinine forms the basis of a renal function test as described by SUNDAL (1935).

The routine clearance methods depend upon an accurate determination of the components of the blood as well as of the urine at definite points of time. Particularly the determination of the *amount* of urine may present considerable difficulty, especially in pediatric practice, and in addition, for example, in obtuse and debilitated individuals. An additional difficulty is the fact that the patients are unable to ingest the necessary amount of fluid — they either refuse, or they vomit the intake immediately. The method employed by SUNDAL consists in giving the patient a certain amount of creatinine perorally, usually 50 mg per kilo of body weight (mean weight compared to height). This results in a rather rapid elevation of creatinine values in the blood to a maximum, in about one hour, followed by a fall along an approximately straight line (Fig. 1), with the fall dependent upon the capacity of the

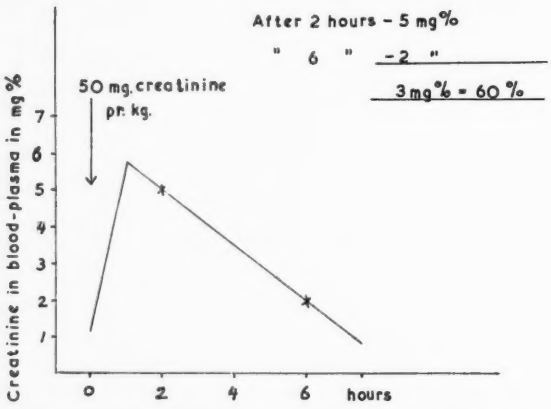

Fig. 1. Creatinine in blood plasma by creatinine tolerance test (after A. SUNDAL).

kidneys to excrete the substance. It is calculated that all creatinine ingested has been eliminated again in the course of seven hours.

The concentration in blood plasma is determined two and six hours after creatinine intake, and registration of the fall in value between these two points of time, in per cent of the first value, gives an expression of the renal function of the patient. SUNDAL assessed the normal figures in children at 40—58 per cent. In a subsequent paper these figures have been changed as follows: definitely pathological values below 45 per cent, doubtful between 45 and 55 per cent.

The creatinine tolerance test has been introduced at the University Pediatric Hospital as a routine renal function test. The method is easily carried out and avoids all difficulties with regard to determination of the amount of urine. The following procedure is employed here:

At 7 a.m. the patient is given 50 mg creatinine per kg of mean body weight, dissolved in a little syrup or similar beverage. At 9 a.m. and again at 1 p.m. a blood sample is collected for determination of creatinine. If a blood urea clearance test is made simultaneously, this is coordinated so that the blood samples for determination of urea clearance and the initial determination of creatinine are both collected at 9 a.m. After all samples have been collected for blood urea clearance, the patient is given a light breakfast, and after the last sample of blood has been collected, dinner as usual.

Results

A creatinine tolerance test has been carried out on 47 patients suffering from renal disease. In order to secure a normal material, the test has been performed also on a number of children with healthy kidneys; and for each

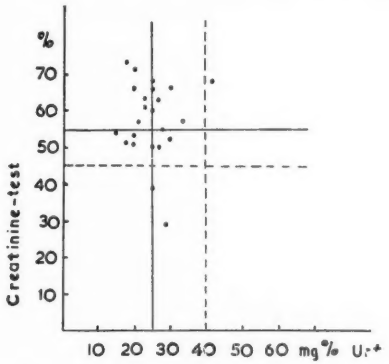

Fig. 2. Comparison between creatinine tolerance and blood urea in 23 children with healthy kidneys.

patient with a renal lesion, an endeavour has been made to find one of the same sex and age with healthy kidneys.

(a) *Thirty control patients* have been examined, and in these a somewhat wider variation has been found than originally stated by SUNDAL, viz. 29—75 per cent. Two patients showed low values, i.e. 29 and 39 per cent. Otherwise, however, they had no signs of renal impairment, and had normal blood urea and urea clearance. In the remaining children with healthy kidneys the lowest value was 49 per cent, and twenty of the thirty control patients had more than 55 per cent. The mean value found (including the lowest two values) was 59 per cent. All these patients had normal urine, normal blood urea, by test performed in 23 patients, and normal urea clearance in the 19 patients on whom this latter test has been carried out.

On 47 patients with renal disease the creatinine tolerance test has been carried out 63 times in all, having been carried out several times on some patients. The diagnosis in the majority of these patients was acute or subacute nephritis, in some hydronephrosis, or other anomalies of the urinary tract, and in these the creatinine tolerance test showed values from 10 to 76 per cent.

(b) *Patients without renal failure.* Thirty-two patients had no clinical renal insufficiency, and showed values ranging from 46 to 76 per cent. In a couple of cases somewhat lower urea clearance than normal was found, probably due to errors in technique, not to reduced renal function. The blood urea values were normal in all cases, and neither did these cases present signs of renal failure.

(c) *Patients with renal failure.* Fifteen patients had a manifest clinical renal failure in a higher or lower degree, with hypo- or isostenuria, high blood urea, decreased urea clearance and/or poor contrast excretion by urography. Crea-

TABLE 1

Creatinine tolerance test compared with blood urea and urea clearance in 15 patients with clinical renal failure.

Case	Age, years	Clinical diagnosis	Creat. test, %	Ur^+ in blood, mg %	Ur^+-clear., %	Case	Age, years	Clinical diagnosis	Creat. test, %	Ur^+ in blood, mg %	Ur^+-clear., %
1. KA	13/12	Renal acidosis	36	50	—	7. HA	7	Hydro-nephrosis	66	40	77
			42	50	—	8. SB	3	Neuro-blastoma	34	146	—
			47	33	26				33	96	—
2. LEH	3	Nephrit. chr.	44	44	—	9. TF	5	Dysplasia renis	51	45	—
			58	23	48						
			53	15	54						
			10	62	20	10. HH	10/12	Hydro-nephrosis	37	83	—
3. Aa.H	13	Nephrit. chr.	11	138	10						
4. ALH	5 ½	Nephro-calcinosis	16	64	26	11. AKA	4	Nephrit. ac.	36	16	37
									57	16	70
5. TW	2	Nephrit. ac.	49	132	—	12. EH	6	Hydro-nephrosis	26	46	31
			57	27	—						
			56	21	—	13. AAa	2 ½	Malformatio	31	40	59
			55	25	—				17	30	—
6. EH	4 ½	Malformatio	48	32	66	14. KM	14/12	Malformatio	30	51	—
						15. JS	5	Hydro-nephrosis	39	34	72

tinine tolerance test has been performed altogether 26 times on these patients, the result of which is shown in Table 1.

Case 2 (Table 1) has been examined four times in all, showing highly variable values, which correspond very well with the clinical course. He had nephritis of a nephrotic type with edema, in some parts very pronounced. The first examination disclosed low creatinine tolerance, somewhat elevated blood urea, failure of urea clearance test. Excessive albuminuria, slight hematuria, mild hypertonia, widespread edema, reduced function by concentration and dilution tests. Some time later he developed measles, and the situation changed completely. For a time he was very well, without edema and without other symptoms of insufficiency—second and third examinations. He then had a further relapse, however, after an attack of tonsillitis, and for some time he was very wretched—fourth examination.

Cases 6 and 7 (Table 1) showed normal values by creatinine tolerance test as well as blood urea and urea clearance. Nevertheless, they have been included in this material because they showed very poor contrast excretion by urography. In addition, case 6 also had pyuria, albuminuria, and low specific gravity of the urine. During a previous stay in another hospital she had shown urea retention.

TABLE 2.

Creatinine tolerance test and other renal function tests in 7 patients with clinical renal failure and elevated plasma creatinine without tolerance test. Maximum creatinine value is the first creatinine value during tolerance test (after 2 hours).

Case	Age years	Plasma creatinine before test, mg %	Maximal creatinine value, mg %	Creatinine test, %	Ur^+ in blood, mg %	Ur^+-clear., %
AaH	13	4.05	14	11	138	10
ALH	5	2	16.1	16	64	26
TW	2	1.7	7.9	49	139	—
EH	4 ½	1.6	11.8	48	32	66
SB	3	2	8	34	146	—
TF	5	1.45	4.4	51	45	—
EH	6	1.7	5.4	26	46	31

It will be observed in Table 1 that on the whole there is very good correspondence between the various renal function tests, and with the clinical picture otherwise.

In the whole series it appeared from the creatinine tolerance test that the *values reached in the blood after two hours* differed greatly. In no case did this maximum value in children with *healthy* kidneys exceed 11 mg/100 ml, and in three-fourths of the cases it was below 7 mg/100 ml. In several patients *with renal disease*, on the other hand, the maximum value was found to exceed 15 mg/100 ml, in one patient it was 16.1 mg/100 ml. In patients *with* renal failure the maximum value in 9 of 26 tolerance tests was found to exceed 7 mg/100 ml, while in patients *without* renal failure it was found to exceed 7 mg/100 ml in only 7 of 37 tolerance tests. This indicates that a strong elevation in blood creatinine was found in approximately twice the number of tests in patients with renal failure than in patients without renal failure. This also seems to be an important factor, therefore, in the evaluation of renal failure.

It has been claimed (HAUGEN) that *the pure value of the creatinine level in blood plasma without any intake of creatinine* suffices for the evaluation of possible renal failure. In all our patients determination of this value has been carried out — as a rule the day prior to the tolerance test. In the normal material none of these values exceed 1.35/mg/100 ml.

Abnormally high creatinine values were found in the blood of two of the patients with renal disease but no clinical signs of renal insufficiency — one, a boy suffering from leukemia, the other, a girl suffering from glycogenosis — who had 1.5 and 2.25 mg/100 ml respectively. In the remaining 30 patients the highest value was 1.35 mg/100 ml.

Eight of the patients with renal insufficiency had a normal creatinine valu in the blood, while seven had an elevated creatinine concentration (Table 2) All of these seven patients had a very severe renal impairment (thoug 3 of them had only slightly decreased creatinine tolerance test). However, th eight patients who had normal creatinine value in the blood also had a definit clinical renal failure. According to this it appears that a rather severe rena impairment has to be present to react on the creatinine level of the blood. This may be expressed by saying that a pathologically increased creatinin value in blood plasma, without any tolerance test having been performed, i a sign of renal failure. On the other hand, however, normal creatinine valu does not exclude the possible presence of renal failure.

Conclusion

In accordance with our present results, the creatinine tolerance test, as a renal function test, seems to be a simple and reliable method. Furthermore, it has the great advantage of being easily managed. It is intended in the first instance for children, but it should also be suitable for other patients, not least for elderly, more or less debilitated and delicate individuals.

Summary

A renal function test with creatinine tolerance determination has been employed in accordance with a method described by SUNDAL. The method seems to be a simple and reliable method, easily managed, and shows good correspondence with other renal function tests.

Le test de tolérance a la créatinine comme test de fonctionnement rénal.

Le test du fonctionnement rénal, par la détermination de la tolérance à la créatinine, d'après la méthode de Sundal, a été essayé. Cette méthode, d'une utilisation facile, semble à la fois simple et précise. Elle donne des résultats analogues à ceux des autres tests de fonctionnement rénal.

Kreatinintoleranz als Nierenfunktionsprobe.

Eine Nierenfunktionsprüfung mit Kreatinintoleranzbestimmung wurde nach der von Sundal angegebenen Methode durchgeführt. Es scheint sich um eine einfache, zuverlässige und leicht durchführbare Methode zu handeln, die gut mit anderen Nierenfunktionsprüfungen übereinstimmt.

Test de tolerancia a la creatinina como prueba de función renal.

Un test de función renal, en el que se emplea la determinación de la tolerancia a la creatinina, según el método descrito por Sundal, ha sido empleado. El método parece sencillo y seguro. Las manipulaciones son fáciles y los resultados congruentes con los de otras pruebas de función renal.

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Pneumo-Encephalography in a Pediatric Department

Review of 214 Cases

With Special Reference to Brain Atrophy

by J. VESTERDAL, K. E. FOGHT-NIELSEN and G. THOMSEN

In recent years pneumo-encephalography has been used extensively in the Pediatric Department, Rigshospitalet, in collaboration with the Radiological Department, and we now feel that a review of the results is desirable. This review will, owing to the nature of the case material, deal mainly with atrophic processes in the brain and their encephalographic picture and clinical appearance.

We have collected 214 consecutive encephalograms from the period January 1948 to July 1952. This number, however, does not cover all the patients from this period examined by encephalography, in that 46 patients during the same period were transferred from the pediatric to the neurosurgical department for encephalography. These patients were either very young (below 8—10 months of age) so that encephalography could most easily be done neurosurgically by ventricular puncture through the fontanel, or they had increased intracranial pressure so that lumbar encephalography was considered dangerous, or a brain tumour was suspected so that we preferred to transfer the patient without delay to the neurosurgical department.

This paper deals only with the encephalograms done in the pediatric department.

Selection of material. — Encephalography was considered to be indicated in all cases where electroencephalography had shown a focal dysrhythmia, and in all cases where other findings had aroused suspicion of a brain tumour; furthermore, in all cases of epilepsy where a thorough examination had not been done before, and in cases showing sequelae from cranial lesions or of intracranial hemorrhage. Finally, encephalography was done in a rather large number of cases where no operable disease was suspected but where an attempt to chart the brain lesion was desirable. To this group belong a number of children with cerebral palsy and with oligophrenia. It appears from this that a great number of our cases are specially selected.

Technique. — We have used the usual procedure for lumbar puncture, as described by Caffey. For anesthesia we have in the course of time used various agents: ether, phenobarbital, chloral hydrate, avertin (tribromoethanol). We now use ether anesthesia after premedication with phenobarbital and atropin.

Complications. — Serious complications were seen in one case only: this infant who was 5 months old, the youngest in our series, had a severe atrophy of the brain. She got an intestinal infection with a malignant Coli bacillus (strain O 55 of Kauffmann), developed a pneumonia and died two days after the encephalography. As this fatality can only questionably be attributed to the encephalography, it seems justifiable to say that the procedure is practically without risk.

In all the other cases no complications were seen, except for some headache and a moderate rise of temperature (seldom above 38.5° C) which generally subsided in a couple of days.

Case material

In Table 1 the case material has been divided into various groups on the basis of clinical symptoms. A division into well-defined nosological entities would, of course, have been better, but at the present stage of our knowledge this is impossible in the majority of the cases. The greater part consists of children with epilepsy, oligophrenia, cerebral palsy, and combinations of these symptoms (some of the patients in the group called spastic diplegia or tetraplegia had also oligophrenia or convulsions). Patients with one-sided symptoms (hemiplegia or jacksonian epilepsy) have been placed in one group. The remaining cases form a number of groups which are either too small or too heterogeneous to allow of general conclusions, and therefore we will consider mainly the first large groups.

TABLE 1

Case material.

Symptoms	No. of cases
1. Epilepsy	30
2. Oligophrenia	30
3. Oligophrenia + epilepsy	25
4. Spastic paraplegia or tetraplegia	58
5. Hemiplegia or jacksonian seizures	23
6. Choreoathetosis or ataxia	7
7. Progressive encephalopathy	8
8. Large hydrocephalic head	5
9. Miscellaneous	28

TABLE 2

Electroencephalographic findings in the larger clinical groups.

	Total	Diffuse dys-rhythmia	Focal dys-rhythmia	Normal	Not examined
1. Epilepsy	30	15	9	3	3
2. Oligophrenia	30	1	1	15	13
3. Oligophr. + epilepsy	25	14	1	3	7
4. Parapl. or tetrapl. spast. .	58	13	5	18	22
5. One-sided symptoms . . .	23	3	14	4	2

TABLE 3

Etiological factors in the larger clinical groups.

	Total	Hereditary factors	Infectious prenatal injury	Neonatal asphyxia	Difficult delivery	Prematurity	Hemolytic disease of newborn	Encephalitis	Meningitis	Pertussis	Cranial trauma
1. Epilepsy	30	4			1	1		2			6
2. Oligophrenia	30			6		1	2	5		2	
3. Oligophr. + epilepsy	25	1	1	2	2			2	2		
4. Spastic parapl. or tetrapl. .	58		1	19	6	7	3	2	1		
5. One-sided symptoms . . .	23			5	3					1	

The classification in Table 1 is rather gross. We have tried a further subdivision of the large groups with regard to clinical symptoms, e.g. a classification of the spastic children into those with paraplegia or tetraplegia, or those with or without convulsions or oligophrenia, and similarly a classification with regard to the electroencephalograms (the results of these have been briefly summarized in Table 2), and finally on the basis of etiology (see Table 3), as it is probable that these factors have some correlation with the pneumoencephalographic findings. However, we have to abandon these subdivisions, for the sub-groups worked out too small to permit of any safe conclusions. With regard to the etiology there is, furthermore, some difficulty concerning the question of birth trauma, which will appear from the following.

It is evident that neonatal asphyxia, e.g. caused by aspiration of amniotic fluid, can injure the brain through hypoxia of the brain tissue, but it is also evident that asphyxia may be a symptom of brain damage with depression of the respiratory centre, developed shortly before or during birth and caused e.g. by protracted uterine contractions or anoxemia of the mother. Asphyxia caused in this way may subsequently damage the brain further.

TABLE 4

Classification of pneumo-encephalograms.

	No. of cases
Normal pneumo-encephalogram	62
Brain atrophy	139
Large communicating hydrocephalus	5
Agenesia of the corpus callosum	2
No air in ventricles	6

It is quite conceivable that a difficult delivery may damage parts of the brain without depressing the respiratory centre, so that the infant will be born non-asphyctic but nevertheless with brain lesions which perhaps will not be detected till later in life. Thus, of the 58 spastic children in our series, 19 were asphyctic at birth, while the delivery had been difficult in 6 other cases, and 7 infants were premature (one of these was asphyctic at birth). This is a rather high incidence of birth complications without neonatal asphyxia. FABER, BENDA and other authors have had similar experiences. This supports the view put forward above. Consequently it may be difficult, in cases with unknown etiology, to exclude birth injury as a pathogenetic factor, as this may have passed unnoticed in the neonatal period. We have considered prematurity to be predisposing to brain damage during birth owing to the softness of the skull of the premature infant. It cannot be excluded, however, that in some of these cases an antenatal injury may have caused the prematurity as well as the brain damage.

Results

Before going into details we can say that in no case have we found any brain tumour, subdural hygroma, or any other condition which could be treated neurosurgically. This is, at least partially, accounted for by the fact already mentioned—that all cases suspected of brain tumour were transferred without delay to the neurosurgical department.

A general review of the results is presented in Table 4.

In 6 cases no air-filling of the ventricles was seen. This was probably due to technical errors. Of course it might also be due to obstruction of the passage from the ventricles to the subarachnoid space, but none of these six children had clinical signs of obstructive hydrocephalus. In one of these a ventriculography was performed later in the neurosurgical department, and this revealed a moderate dilatation of the lateral ventricles. In the other five

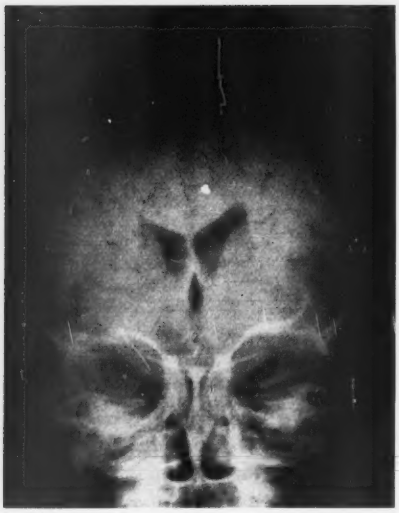

Fig. 1. Very slight enlargement of one lateral ventricle.

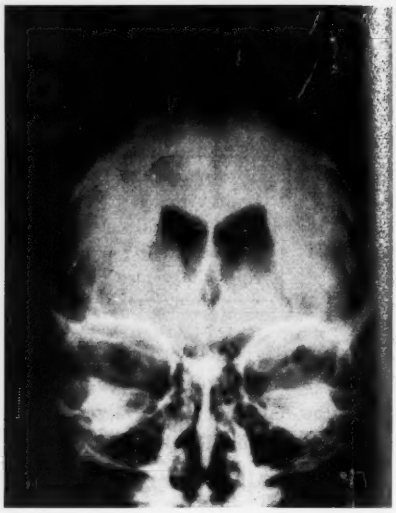

Fig. 2. Enlargement to a medium degree of both lateral ventricles and of III ventricle.

cases the encephalography was not repeated because no operable disease was suspected.

In 5 cases air-filling of only one lateral ventricle was seen. This was in all cases thought to be due to technical errors, as the neurological symptoms were symmetrical. These encephalographies were not repeated, and we have classified the results according to the findings on the air-filled side.

In 62 cases normal encephalograms were found.

Brain atrophy of varying localization and severity was found in 139 cases. The term brain atrophy indicates not only atrophy of previously normally developed parts but also defective development, for it is in most cases impossible, on the encephalogram, to differentiate between these pathological conditions.

On the encephalogram the brain atrophy appeared in most cases as a more or less pronounced enlargement of the lateral ventricles (Figs. 1, 2, 3, and 4). A very slight dilatation may possibly be an artefact, as the brain substance in children is very soft. Sometimes enlargement of the third or fourth ventricle was seen (Figs. 2 and 4). In many cases an increased amount of air was seen on the outside of the brain, most often on the upper convexity: this was frequently associated with increased width of the sulci, indicating cortical atrophy (Fig. 3). In some cases an enlargement of cisterna interpeduncularis or cisterna magna was seen: the latter condition combined with enlarge-

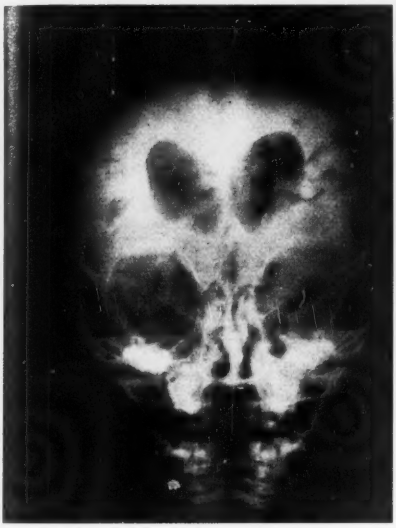

Fig. 3. Severe enlargement of both lateral ventricles. Increased width of the cortical sulci in both frontal regions.

Fig. 4. Enlargement of cisterna magna and IV ventricle. Severe enlargement of both latera ventricles (only the posterior horns are seen)

ment of the fourth ventricle indicating cerebellar atrophy, as pointed out by MURPHY and ARANA (Fig. 4).

We have been very cautious in estimating the size of the basal cisterns: misinterpretations may easily occur, particularly because subdural air is apt to be found below the tentorium. It is important to realize that all subdural air accumulations are to be considered as artefacts, see DAVIDOFF and EPSTEIN. Sometimes even subarachnoidal air accumulations may cause erroneous results (2).

In some cases porencephaly was present, consisting of localized atrophies on the cortical or the ventricular side of the brain substance (Fig. 5).

Even in the cases with great dilatation of the ventricular system the head circumference was normal, or only slightly enlarged, in some cases even diminished, so that microcephaly was present. The combination of microcephaly and dilated ventricles is by some authors called hydromicrocephaly (see MCCLELLAND). In this condition it is evident that the dilatation of the ventricles is caused by a primary atrophy of the brain substance and not by increased intraventricular pressure, in contrast to the cases of marked hydrocephalus with large heads.

Displacement of the ventricles was observed in connection with asymmetrical atrophy, the ventricles being drawn over towards the more atrophic

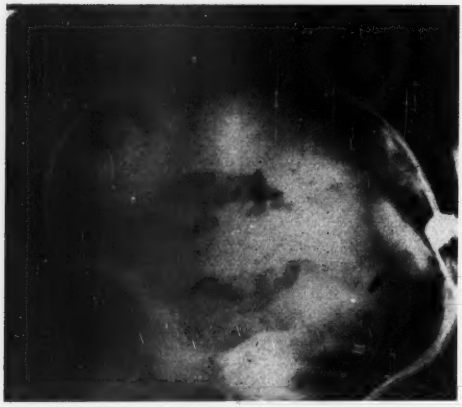

Fig. 5. External porencephaly in the frontal region.

side usually. One case showed a doubtful depression of the posterior horn of one of the lateral ventricles which were otherwise normal. Therefore, a space-occupying lesion above the posterior horn was suspected, but when a bore-hole was made subsequently in the neurosurgical department nothing except doubtful cortical atrophy was found.

Five cases had large hydrocephalus of the communicating type, with large head circumference.

Two cases showed agenesia of the corpus callosum (Figs. 6 and 7). In some cases this malformation may cause no symptoms at all. One of our patients, however, was oligophrenic, and the other one was a dwarf with hypothyroidism, diabetes insipidus and congenital eye abnormalities, but without mental retardation.

We shall now consider each of the major clinical groups in Table 1 and review the encephalographic findings in order to see the connection, if any, between these and the clinical symptoms.

In each group the encephalograms have been classified primarily according to the appearance of the lateral ventricles, as this was found to be the simplest and most unequivocal basis of classification. After this, the other findings (signs of cortical atrophy, enlarged III or IV ventricle, enlarged basal cisterns) have been recorded.

1. Epilepsy. — Of these 30 cases, 21 had grand mal, 7 petit mal, and 2 both grand and petit mal. Cases with jacksonian seizures have been listed in group 5. The electroencephalographic findings and the etiological factors appear from Tables 2 and 3. The pneumo-encephalograms have been summarized in Table 5. In comparison with the following clinical groups the percentage of

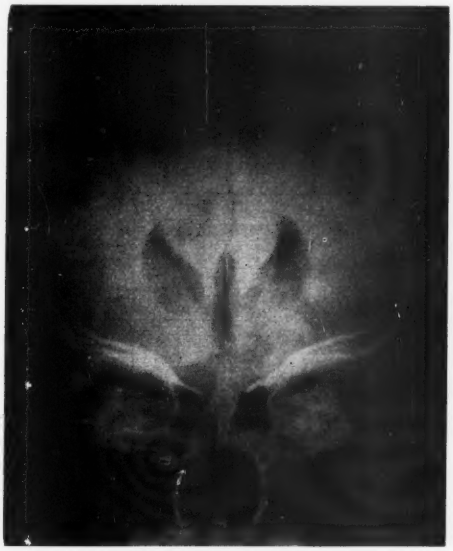

Fig. 6.

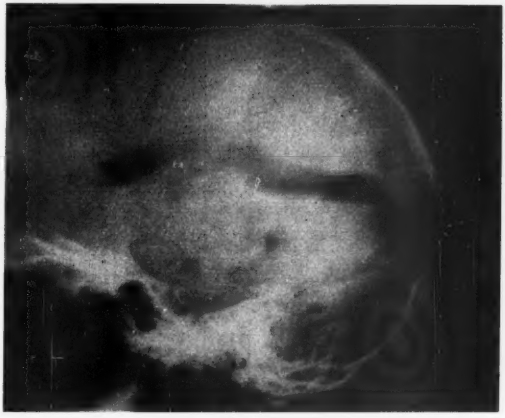

Fig. 7.

Figs. 6 and 7. Agenesia of the corpus callosum.

ormal findings is rather high, and the pathological findings, when present, re only slight. The patients with familial epilepsy had normal encephalo- rams, except one who had a moderate dilatation of the lateral ventricles. No ignificant differences could be found between the clinical, etiological or lectroencephalographical subdivisions.

TABLE 5

In each clinical group the encephalograms have been classified primarily according to the appearance of the lateral ventricles, and afterwards the other findings — enlargement of III or IV ventricle or of the basal cisterns, cortical atrophy, external porencephaly — have been listed (the last subdivisions may overlap).

	Total	Cortical atrophy	III ventr. enlarged	IV ventr. enlarged	Cist. magna enlarged	Cist. interped. enlarged	External porencephaly
1. Epilepsy (30 cases)							
Lateral ventricles normal	23	3	1			1	
Enlargement of lat. ventricles: slight	4						
Enlargement of lat. ventricles: medium	2						
Enlargement of lat. ventricles: severe	0						
No air in ventricles	1						
2. Oligophrenia (30 cases)							
Lateral ventricles normal	15	2				2	
Enlargement of lat. ventricles: slight	3						
Enlargement of lat. ventricles: medium	9	5	4		1	1	2
Enlargement of lat. ventricles: severe	2		1		1	1	
Agenesia of the corpus callosum	1						
3. Oligophr. + epilepsy (25 cases)							
Lateral ventricles normal	8	4					
Enlargement of lat. ventricles: slight	6	3	2				
Enlargement of lat. ventricles: medium	6	3	3			1	
Enlargement of lat. ventricles: severe	5	1					1
4. Spastic paraplegia or tetraplegia (58 cases)							
Lateral ventricles normal	15	4					
Enlargement of lat. ventricles: slight	15	7			1		
Enlargement of lat. ventricles: medium	20	8	6	1	2		
Enlargement of lat. ventricles: severe	6		1		1		
No air in ventricles	2	1					
5. One-sided symptoms (23 cases)							
Lateral ventricles normal	5						
Enlargement of lat. ventricles: slight	6	3	1				(asym.: 5)
Enlargement of lat. ventricles: medium	8	2					(— : 8)
Enlargement of lat. ventricles: severe	1		1				
Internal porencephaly	1						(— : 1)
No air in ventricles	2						

2. Oligophrenia. — Of these 30 cases, 5 also had muscular hypotonicity, 6 had an inconspicuous spastic paraplegia, 2 had speech defects and 2 were probably deaf. In many cases severe atrophy of the brain was seen (Table 5), appearing as an enlargement of the lateral ventricles or sometimes of the third ventricle, or as cortical atrophy. One case showed agenesia of the corpus callosum. Many cases revealed a disproportion between the degree of oligophrenia and the severity of the brain atrophy visible on the encephalogram. No significant difference was found between the clinical, etiological or electroencephalographic subdivisions.

3. Oligophrenia combined with epilepsy. — Of these 25 cases, 2 also had muscular hypotonicity, 2 had an inconspicuous spastic paraplegia, and 2 were probably deaf. Six had petit mal, 17 grand mal, and 2 both grand and petit mal. The encephalographic findings (Table 5) were very much like those in the preceding group, except that the percentage of normal encephalograms was lower in group 3. There was no significant difference between the clinical, etiological or electroencephalographical subdivisions.

4. Spastic paraplegia or tetraplegia. — Of these 58 cases, 24 had paraplegia and 34 tetraplegia. Two in the first group and 4 in the second had some asymmetry of the symptoms. At least 29 of the patients were oligophrenic (the exact number cannot be stated, because of the difficulty of estimating the mental development in these handicapped children). Three had epilepsy and 3 had jacksonian seizures, but as these were only minor symptoms of the disease these patients have been classified in this group. Besides the spasticity, 13 had choreoathetosis and 4 had ataxia. Generally, brain atrophy of varying degree was found, but sometimes the findings were normal (Table 5). On the whole the results were similar to those from the two preceding groups, and there was no significant difference between the clinical, etiological or electroencephalographical subdivisions. Of the 6 patients with asymmetrical symptoms, 2 had asymmetrical brain atrophy.

5. One-sided symptoms. — Of these 23 cases, 11 had hemiplegia, 9 had jacksonian seizures, and 3 had both symptoms; 5 had also common epileptic seizures; 4 were mentally retarded. The encephalographic findings are shown in Table 5. In about 60 per cent the brain lesion visible on the encephalogram was asymmetrical while only 10 per cent of the cases in all the other clinical groups had asymmetrical atrophy. Except in one case, the atrophy was most pronounced in the hemisphere which was contralateral to the clinical symptoms. In one case of hemiatrophy of the brain a subdural hematoma was suspected, but this diagnosis was not verified when a bore-hole was made afterwards in the neurosurgical department.

TABLE 6

Encephalographic findings in (A) patients with history of neonatal asphyxia, difficult delivery, prematurity (prenatal injury cannot be excluded in these cases), or hemolytic disease of the newborn, (B) patients with history of encephalitis, meningitis, or cerebral vascular lesions in pertussis, and (C) the remaining cases, except those with large hydrocephalus or progressive encephalopathy. The groups have been subdivided as in Table 5.

	Total	Cortical atrophy	III ventr. enlarged	IV ventr. enlarged	Cist. magna enlarged	Cist. interped. enlarged	External porencephaly
A. 69 cases with paranatal injury							
Lateral ventricles normal	22	4				1	
Enlargement of lat. ventricles: slight	12	3					1
Enlargement of lat. ventricles: medium	25	12	5	1	2	2	1
Enlargement of lat. ventricles: severe	6		1				1
Internal porencephaly	2						
No air in ventricles	2						
B. 20 cases with postnatal infectious injury							
Lateral ventricles normal	7						
Enlargement of lat. ventricles: slight	4	2	1				
Enlargement of lat. ventricles: medium	6	1	2				
Enlargement of lat. ventricles: severe	3						1
C. Remaining cases (112)							
Lateral ventricles normal	52	10	2			2	
Enlargement of lat. ventricles: slight	27	8	3				
Enlargement of lat. ventricles: medium	22	8	8				
Enlargement of lat. ventricles: severe	5	1		1	2	1	
Internal porencephaly	1						
Agenesia of the corpus callosum	2						
No air in ventricles	3						

The remaining clinical groups (see Table 1) are, as mentioned before, either too small or too heterogeneous to permit of detailed analysis. We shall only mention that the patients with progressive encephalopathies (mainly abiotrophies of the Neel–Einarsson type or similar conditions) and those with choreoathetosis or ataxia had either normal encephalograms or brain atrophy of the same character as seen in groups 2, 3 and 4. Curiously enough, cerebellar atrophy was not clearly visible in the cases of ataxia.

In order to see if paranatal brain injuries or postnatal infective lesions may cause any characteristic features on the encephalogram, we have divided the

whole series, except the cases of large hydrocephalus and progressive encephalopathy, into three groups according to etiology without regard to clinical symptoms (Table 6): (A) patients with a history of neonatal asphyxia, difficult delivery, prematurity (prenatal injury cannot be excluded in these cases), or hemolytic disease of the newborn, (B) patients with a history of encephalitis, meningitis, or cerebral vascular lesions occurring during whooping cough, and (C) the remaining cases. A comparison between the encephalograms from these three groups shows that the percentage of normal pictures was 25 in group (A) and 35 in (B) and (C). The brain atrophy, when present, was of the same character in all three groups.

Discussion

GUTTMANN, MCCLELLAND, MURPHY and ARANA, and other authors have published series of cases with severe brain atrophy of various sorts visible on the pneumo-encephalogram, associated with oligophrenia, cerebral palsy, hemiplegia, epilepsy and other clinical symptoms, but they do not give any statements about the frequency of these findings within each clinical group. BRINES and LORD, FORD, DAVIDOFF and EPSTEIN, and CAFFEY have found that there is often a discrepancy between the severity of the clinical symptoms and the encephalographic findings in such patients. BENDA has made a thorough study of oligophrenia and cerebral palsies, mainly from a patho-anatomical point of view. Some of his cases had been examined by encephalography, and generally brain atrophy was found, but apparently one has to be very cautious in interpreting the encephalogram. Some patho-anatomical conditions may be invisible on the encephalogram, e.g. porencephalic cysts, as these only in some cases communicate with the ventricles or the subarachnoid space.

In our series, brain atrophy was present in a great number of patients, but the character of the atrophy was only to a small degree correlated with the nature of the clinical symptoms, in so far as the same encephalographic pictures were found in cases with the following symptoms: oligophrenia, spastic paraplegia or tetraplegia, choreoathetosis, ataxia, and combinations of these symptoms with each other or with epilepsy. None of these symptoms was accompanied by any characteristic feature on the encephalogram.

Generally, severe atrophy visible on the encephalogram was associated with severe oligophrenia or cerebral palsy or, in case of hemiatrophy, with severe hemiplegia. In many cases, however, similar clinical conditions were associated with normal encephalograms. Conversely, a combination of slight clinical symptoms with a rather severe brain atrophy was seen, but this was rather infrequent. Thus we have seen a $5\,^1/_2$ year old girl with a moderate

spastic paraplegia and in IQ of 124, who had an enlargement to a medium degree of the lateral ventricles but no signs of cortical atrophy. Of course this IQ may be reduced, for if no cerebral damage had existed she may have had an IQ of e.g. 160.

That severe clinical symptoms may be associated with a normal encephalogram in some cases and with severe atrophy visible on the encephalogram in other cases in easily understood when one realizes that degenerated nerve cells in some cases are replaced with glial tissue and in others not. Only in the latter cases the atrophy will be visible on the encephalogram, while in the former a discrepancy will be found between the clinical and encephalographic findings. This, of course, is very puzzling and limits the diagnostic value of encephalography in the clinical groups in question.

The cases with the greatest accordance between the clinical and encephalographic findings were those with one-sided symptoms. Here asymmetrical atrophy was found in 60 per cent of the cases, while an asymmetrical picture was seen in only 10 per cent of the rest of the material. With a single exception the most severe atrophy was contralateral to the clinical symptoms.

In a great number of cases the brain atrophy was associated with electroencephalographic abnormalities, either diffuse or focal. In some cases cortical atrophy was found at the site of the electroencephalographic foci, but in other cases nothing particular could be found here. In fact, in most cases no correlation existed between the electroencephalogram and the pneumoencephalogram.

It is conceivable that a single pathogenetic factor, e.g. birth trauma, might cause some characteristic traits which were seen repeatedly on the encephalograms, in spite of the variety of the clinical symptoms which arise apparently in a rather haphazard way. Such traits, however, were not recognizable when each clinical group was regarded separately, nor were they obvious when the whole material was considered. Thus, when the patients with a history of paranatal injury were compared with those with postnatal infective lesions and with the remaining cases, only a slight difference was found, the percentage of normal encephalograms being slightly smaller in the first group than in the two others (25 as against 35 per cent). The brain atrophy, when present, was of the same character and degree in these three groups. Although one has to be cautious in drawing any statistical conclusions from a material which is in some ways selected, it seems justifiable to conclude that no specific encephalographic pictures are associated with the brain lesions following paranatal injury or postnatal infections.

The brain lesions arising from neonatal asphyxia or difficult delivery or prematurity are, except for hemiatrophy and porencephaly, generally so diffuse that one could hardly imagine that they would be the result of vas-

cular lesions; more likely hypoxia of the brain tissue is the cause. BENDA, however, holds the view that the atrophy of the brain substance round the ventricles, visible as an enlargement of these, may be due to stasis in the great cerebral vein of Galen and perivascular hemorrhages round this vein and its tributaries, occurring during birth.

As to the value of encephalography in childhood we are, in accordance with other authors, of the opinion that frequently this procedure is of limited usefulness in the diagnosis of cerebral palsy and oligophrenia, and generally without value with regard to the therapy of such cases. However, as long as our knowledge about the brain lesions in these conditions is rather limited, we consider encephalography to be of scientific interest here, the more so because cerebral palsy in recent years has been a field attracting increasing interest. We share the general opinion that encephalography, apart from conditions suspect of brain tumour, is indicated in epilepsy, sequelae of cranial lesions or of intracranial hemorrhage, and in doubtful neurological cases. In children of the last category, e.g. small children with vague symptoms such as muscular hypotonicity, the encephalogram may be valuable in demonstrating whether or not brain atrophy is the cause of the symptoms.

Summary

The authors review 214 consecutive encephalograms from children mostly suffering from epilepsy, oligophrenia, cerebral palsy, or various combinations of these symptoms. Sixty-two patients had normal encephalograms, while almost all the others had more or less pronounced brain atrophy. Except in the cases with one-sided symptoms, no conspicuous correlation existed between the nature of the symptoms or the electroencephalograms, and the character of the brain atrophy, except that most epileptics had normal pictures. Very often there was a disproportion between the severity of the clinical symptoms and the degree of brain atrophy visible on the encephalogram. In 60 per cent of the patients with hemiplegia or jacksonian seizures asymmetrical atrophy of the brain was founded. Agenesia of the corpus callosum was found in two cases. That no disease which could be treated neurosurgically was found in this series is, at least partially, accounted for by our practice of immediate transfer to the neurosurgical department of all cases suspected of brain tumour.

Pneumoencéphalographies dans un service de pédiatrie. Revue de 214 cas, avec remarques au sujet de l'atrophie cérébrale.

Les auteurs passent en revue 214 encéphalogrammes faits chez des enfants souffrant d'épilepsie, d'oligophrénie et paralysies cérébrales ou de l'association de ces 3 symptômes. 62 malades avaient des images normales, tandis que la plupart des autres présentaient une atrophie plus ou moins prononcée. En dehors des cas avec une atteinte unilatérale, il n'existe pas de relation entre la nature des symptômes, ou les tracés EEG et les caractères de l'atrophie cérébrale, sauf si l'on tient compte que la plupart des épileptiques avaient des images normales. Souvent les auteurs constatèrent une disproportion entre la sévérité des signes cliniques et l'importance

de l'atrophie cérébrale sur les clichés. Dans 60 % des cas d'hémiplégie ou d'épilepsie jacksonienne, on trouva une assymétrie du cerveau. Dans 2 cas il y avait une agénésie du corps calleux. Le fait de n'avoir pas trouvé de cas justiciable de la neuro-chirurgie tient à notre habitude d'adresser dans ces services tous les cas suspects de tumeur cérébrale, et ceci immédiatement.

Pneumoencephalographie an einer Kinderabteilung. Bericht über 214 Fälle. Mit besonderer Berücksichtigung der Gehirnatrophie.

Die Autoren berichten über 214 aufeinanderfolgende Encephalogramme bei Kindern, von denen die meisten an Epilepsie, Oligophrenie, cerebraler Lähmung oder verschiedenen Kombinationsformen dieser Symptome litten. 62 Patienten hatten ein normales Encephalogramm, während alle anderen mehr oder minder hervortretende Zeichen einer Gehirnatrophie zeigten. Mit Ausnahme der Fälle mit einseitigen Symptomen besteht keine augenfällige Korrelation zwischen der Natur dieser Symptome, oder den Encephalogrammen, und dem Charakter der Gehirnatrophie, ausgenommen, daß die meisten Epileptiker normale Bilder boten. Sehr oft lag ein Mißverhältnis zwischen der Schwere der klinischen Symptome und dem im Encephalogramm sichtbaren Ausmaß der Gehirnatrophie vor. In 60 % der Fälle von Hemiplegie und Jacksonepilepsie wurde eine asymmetrische Gehirnatrophie gefunden. Agenesie des Corpus callosum wurde in 2 Fällen festgestellt. Daß keine für eine neurochirurgische Behandlung geeigneten Fälle in dieser Untersuchungsreihe aufgedeckt wurden liegt vorwiegend daran, daß üblicherweise alle auf Gehirntumoren verdächtigen Fälle sofort der neurochirurgischen Abteilung überwiesen werden.

Neumo-encefalografía en un departamento pediátrico. Repaso de 214 casos con especial referencia a la atrofia del cérebro.

Los autores repasaron 214 encefalogramas consecutivos de niños que en su mayor parte sufrían de epilepsia, oligofrenia, parálisis cerebral o varias combinaciones de estos síntomas. 62 pacientes presentaron encefalogramas normales, mientras que casi todos los demás mostraron una atrofia del cérebro más o menos pronunciada. Excepto en los casos con síntomas unilaterales no existía ninguna correlación clara entre la naturaleza de los síntomas o los encefalogramas y el carácter de la atrofia del cérebro, exceptuando el que el mayor número de epilépticos mostraba figuras normales. Muy amenudo apareció una disproporción entre la severidad de los síntomas clínicos y el grado de la atrofia cerebral visible en el encefalograma. En el 60 por ciento de los pacientes con hemiplegia o síntomas de Jackson se encontró una atrofia asimétrica del cérebro. Se encontró agenesia del cuerpo calloso en dos casos. El que no se encontrara en estas series ninguna enfermedad que podía ser tratada neuroquirurgicamente es debido, por lo menos en parte, a nuestra costumbre de traslado al departamento de neurocirugía de todos los casos sospechosos de tumor cerebral.

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Resultats d'une Étude de Sérums d'Enfants Atteints de Néphrose Lipoïdique par la Méthode de Micro-Électrophorèse sur Papier

par C. HOOFT et R. CLARA

Les sérums de 9 enfants présentant les signes cliniques de néphrose lipoïdique ont été étudieés par la méthode de micro-électrophorèse sur papier, mise au point par TURBA et ENENKEL, CREMER et TISELIUS, et DURRUM. L'appareil que nous avons employé est mis sur le marché par la firme Bender et Hobein, sous le nom d'*Elphor-H*.

L'âge de nos patients varie entre 11 mois et 13 ans. A une exception près, ces malades présentaient la symptomatologie des néphroses lipoïdiques pures. Dans tous ces cas, les symptômes cliniques de la néphrose lipoïdique se sont manifestés comme les tout premiers signes de leur maladie; la néphrose n'était donc pas secondaire à un état pathologique préalable. Chez 8 enfants, le taux d'urée et le clearance de l'urée étaient normaux. Une hématurie franche n'a pas été observée. Comme il est fréquemment constaté dans la néphrose lipoïdique, nous avons trouvé de temps en temps quelques rares érythrocytes à l'examen microscopique du sédiment urinaire. Un de nos malades présentait les signes manifestes d'une néphrite aiguë.

Avant de donner les résultats de nos examens, nous jugeons utile de discuter de la valeur et des possibilités de la micro-méthode que nous avons employée.

La méthode de micro-électrophorèse sur papier est basée sur la combinaison de la chromatographie et de l'action d'un champ électrique. Cette méthode étudiée par HAUGAARD et KRONER (1948), WIELAND et FISCHER (1948) pour les acides aminés a été appliquée par DURRUM (1950), TURBA et ENENKEL (1950), CREMER et TISELIUS (1950) à l'étude des protéines.

Nous avons utilisé la méthode colorimétrique directe, préconisée par GRASSMANN, HANNIG et KNEDEL, qui emploient comme réactif une solution saturée d'admidoschwarz dans un mélange méthanol-acide acétique (9/1).

Tous les auteurs sont d'accord pour reconnaître que les résultats obtenus par la micro-électrophorèse sur papier sont tout à fait comparables à ceux obtenus par la macro-méthode.

Les différences retrouvées avec la méthode de TISELIUS varient d'après les auteurs: d'après GRASSMANN, WIEDEMANN et SCHULTZE il n'y aurait qu'un léger écart pour les différentes fractions; une déviation moyenne pour l'albumine de 1,2%; pour la globuline α_1 de 0,6%, pour la globuline α_2 de 0,7%, pour la globuline β de 0,6% et pour la globuline γ de 1,0%. Au contraire OTT, HUBER et KÖRVER trouvent des valeurs plus élevées pour l'albumine (+ 7%) et la globuline γ (+ 1%) et des valeurs toujours moins élevées pour la globuline β (− 5%).

Il est plus que probable que les valeurs moins élevées de la globuline β trouvées avec la méthode de micro-électrophorèse sur papier se rapprochent davantage de la réalité que les valeurs plus élevées obtenues par la macro-électrophorèse. En effet, ZELDIS et ALLING, KUNKEL et AHRENS, LONGSWORTH et MACINNES ont pu démontrer qu'avec des méthodes optiques l'indice de réfraction de la globuline β est fortement influencé par les lipoïdes.

Principalement pour l'étude des sérums de malades atteints de néphrose lipoïdique, les valeurs obtenues pour la globuline β par la micro-électrophorèse sur papier sont plus précises que celles obtenues par les méthodes optiques. Un autre avantage de la micro-méthode sur papier est la possibilité de différencier la globuline α, ce qui n'est pas toujours possible avec l'appareil de TISELIUS ou l'appareil de Kern d'après LABHART et STAUB.

Cette différenciation est très importante dans la néphrose lipoïdique où l'α_2 est toujours fortement augmentée tandis que l'α_1 reste normale.

Nous avons examiné les sérums de 17 enfants bien portants avec l'appareil Elphor-H pour établir la moyenne des valeurs normales — (tampon de Michaëlis, pH = 8,6 et $\mu = 0{,}1$). Nos résultats concordent approximativement avec ceux obtenus par GRASSMANN et HANNIG chez 25 adultes (tampon Véronal-Véronal sodé).

	Albumine	α_1 %	α_2 %	β %	γ %
GRASSMANN & HANNIG . . .	61,3	4,1	8,1	11,0	15,5
Moyennes de 17 enfants normaux	63,8	3,5	10	10,1	12,6

Nous avons déterminé le taux des protéines totales par le procédé au sulfate de cuivre de PHILLIPS, VAN SLYKE c. s. Cette méthode de dosage est rapide et de manipulation simple. La comparant à d'autres méthodes, les résultats en sont très satisfaisants. La méthode est plus précise que la réaction au biuret et la détermination interférométrique, surtout pour des sérums pathologiques. D'après VAN DOMMELEN, PHILLIPS et VAN SLYKE et c. s., LOWRY et HUNTER, MEYER et c. s., il n'y aurait qu'une différence moyenne de 0,24 à 0,26 avec la méthode de Kjeldahl.

Observations personnelles dans la néphrose lipoïdique

Les valeurs obtenues chez nos 9 malades sont résumées dans le Tableau 5. Notons dès à présent que nous n'avons pas tenu compte des résultats obtenus au cours d'un traitement à l'A.C.T.H. ou peu après la transfusion de grandes quantités d'albumine. De même, nous ne mentionnons pas les résultats que nous avons pu obtenir chez quelques malades peu de temps avant une guérison spontanée. Ces données-là feront l'objet d'une autre communication.

Dans tous les cas le taux des protéines totales est très bas, en moyenne 4,15 g% (min. 3,1 g%–max. 5,35 g%). Le taux de l'albumine est également très bas, en moyenne 34,6% ou 1,48 g% (min. 15% ou 0,6 g%, max. 50% ou 2,9 g%).

Les valeurs relatives de la globuline α_1 sont un peu au-dessus de la normale, en moyenne 4,8% (min. 1,1%, max. 11%), les valeurs absolues sont diminuées, en moyenne 0,2 g% (min. 0,05 g%, max. 0,4 g%).

La globuline α_2 est toujours très élevée, en moyenne 34,8% ou 1,41 g% (min. 20% ou 0,8 g%, max. 49% ou 2,4 g%).

Les valeurs relatives de la globuline β dépassent en général les valeurs normales, en moyenne 18,2% (min. 10,1%, max. 33%); les données quantitatives sont à peu près normales, en moyenne 0,75 g% (min. 0,32 g%, max. 1,26 g%).

La globuline γ montre dans la plupart des cas des valeurs relatives un peu en dessous de la normale, en moyenne 7,5% (min. 1,4%, max. 14,7%); mais les données absolues sont toujours nettement inférieures à la normale, en moyenne 0,31 g% (min. 0,05 g%, max. 0,68 g%).

Ces constatations confirment les données de la littérature à ce sujet qui furent établies pour la première fois en 1939 par LONGSWORTH et MACINNES.

Dans la néphrose lipoïdique, la répartition des différentes protéines sériques donne une image typique: diminution du taux de l'albumine et de la globuline γ; augmentation constante de l'α_2 et dans la plupart des cas, une augmentation de la β et parfois de l'α_1. Les valeurs relatives et absolues de ces différentes fractions peuvent cependant varier considérablement si on compare les résultats obtenus chez plusieurs malades. Ces divergences ont été notées par tous les auteurs (LONGSWORTH et MACINNES, MALMROS et BLIX, FARNSWORTH et RUPPENTHAL, KROPP et ALTHOFF, ROUTH, KNAPP et KOBAYASHI, FISCHEL et STEINMAN).

En groupant les résultats d'après le tableau clinique ou d'après la teneur du sérum en protéines totales, nous avons tâché de trouver une explication de ces variations.

Aspect clinique

Si nous groupons les valeurs relatives et absolues des différentes fractions protéiques, suivant l'état d'œdème il nous est difficile d'en déduire des conclusions définitives.

En considérant la moyenne des résultats, on a l'impression que les valeurs relatives et absolues de l'albumine diminuent avec la généralisation des œdèmes, tandis que les valeurs de l'α_2 augmentent (Tableau 2). Les différences pour l'albumine, la globuline α_2 et la globuline γ sont très nettes, si l'on compare les états cliniques extrêmes.

La valeur de cette constatation diminue sensiblement, si on tient compte du nombre relativement restreint d'examens pratiqués dans la période d'œdème généralisé avec ascite.

En outre, les écarts importants entre minima et maxima notés pour chaque état (œdème ou absence d'œdème) ne permettent pas de dégager une relation entre les modifications pathologiques des différentes fractions et l'aspect clinique.

Protéines totales

Dans un autre tableau, nous avons groupé les valeurs relatives des différentes fractions en fonction du taux des protéines totales. D'après les moyennes obtenues, les pourcentages de l'albumine et de la globuline α_2 par rapport aux protéines totales vont respectivement en croissant et décroissant (Tableau 3). Mais, de nouveau, les minima et maxima varient tellement dans chaque sous-groupe analysé qu'il est impossible de trouver une corrélation entre la répartition des différentes fractions et la teneur en protéines totales de sérum. Nous retrouvons souvent les mêmes pourcentages des différentes fractions qu'il s'agisse d'une protéinémie totale très élevée ou d'une protéinémie totale très basse.

Relation entre les fractions protéiques

Quelques auteurs ont tâché de trouver une corrélation entre les différentes fractions. Ainsi ROUTH, KNAPP et KOBAYASHI ont suggéré une relation entre l'abaissement de l'albumine et le taux élevé de l'α_2 dans la néphrose lipoïdique. Cependant ce fait n'est pas nettement établi dans leur communication. On peut faire ressortir cette relation en portant sur un graphique les variations des 2 fractions: albumine et globuline α_2 (Fig. 1).

La droite obtenue de cette manière montre que les deux fractions varient en sens inverse. Cette corrélation linéaire entre la globuline α_2 et l'albumine semble être d'ailleurs une règle générale. Sur la même ligne, nous retrouvons les sérums normaux et les sérums d'autres états pathologiques que la néphrose lipoïdique.

TABLEAU 1

Résultats de l'examen électrophorétique du sérum de 9 malades, atteints de néphrose lipoïdique (la méthode d'Elphor).

Date	Prot. tot. %	Alb. %	α_1 %	α_2 %	β %	γ %	Alb. g %	α_1 g %	α_2 g %	β g %	γ g %
Jacqueline C., 3 ans.											
17.3 52	5,0	26,1	1,93	39,6	22,22	10,15	1,3	0,1	2,0	1,11	0,49
24.3 52	4,8	32,1	5,0	31,2	21,8	9,5	1,54	0,25	1,5	1,04	0,47
4.4 52	5,0	42,6	5,2	26,5	14	11,7	2,2	0,25	1,27	0,7	0,58
25.4 52	5,0	42,6	5,8	26,1	17,55	7,95	2,2	0,29	1,23	0,88	0,4
16.5 52	3,9	37	4,5	34,7	15,1	8,7	1,44	0,18	1,35	0,59	0,34
26.9 52	6,1	47,1	4	23,2	20,7	5	2,87	0,25	1,41	1,26	0,31
Ghislain D. B., 4 ½ ans.											
24.3 52	4	15,55	5,6	50,3	21,1	7,45	0,62	0,22	2,01	0,85	0,3
31.3 52	3,4	15,75	1,58	49,1	23,87	9,7	0,54	0,05	1,67	0,81	0,33
7.4 52	3,9	14,9	2,45	58,2	16,15	8,3	0,58	0,1	2,27	0,63	0,32
8.5 52	4,25	15,5	3,4	56,6	18,2	6,3	0,66	0,15	2,4	0,77	0,27
3.7 52	3,5	25,5	4,9	45	17,1	7,5	0,89	0,17	1,58	0,6	0,26
5.8 52	3,9	30	5	44,5	14,7	5,8	1,17	0,2	1,73	0,57	0,23
9.9 52	3,5	26,17	5,24	43	16,05	9,54	0,92	0,18	1,51	0,56	0,33
17.11 52	4,15	33,0	4	45	16	2	1,37	0,17	1,87	0,66	0,08
Maurits L., 2 ans et 7 mois.											
26.9 52	3,9	18,9	1,1	52	23,2	4,8	0,74	0,04	2,0	0,9	0,19
13.10 52	3,15	18,9	6,1	63,5	10,1	1,4	0,5	0,19	2,0	0,32	0,05
19.10 52	3,5	18,4	3,1	48,4	21,1	8	0,64	0,11	1,7	0,74	0,31
27.10 52	3,15	21,6	5,1	54,5	14,2	4,6	0,68	0,16	1,72	0,45	0,14
17.11 52	3,9	30,3	6,2	38,2	18,3	7,0	1,2	0,28	1,5	0,7	0,27
1.12 52	3,9	37,3	4,4	39,3	13,1	5,9	1,45	0,17	1,56	0,5	0,22
Jennifer R., 1 an.											
30.4 52	4,2	44	6	29,5	14,5	6	1,85	0,25	1,24	0,61	0,25
7.5 52	3,9	32,7	11	30,4	18,7	7,2	1,27	0,43	1,19	0,73	0,28
12.5 52	3,9	32,7	4,6	38,3	16,6	7,8	1,28	0,18	1,48	0,65	0,31
14.5 52	4,25	35,5	6,4	37,1	14,5	6,5	1,51	0,27	1,56	0,63	0,28
16.5 52	4,6	36	5,2	34,7	18	5,9	1,66	0,24	1,6	0,83	0,27
19.5 52	4,6	37,9	3,3	34,5	16,8	7,5	1,75	0,16	1,6	0,78	0,31
21.5 52	3,9	37,85	3,8	35,65	16,7	6	1,47	0,15	1,39	0,65	0,24
23.5 52	3,9	40	1,43	29,7	21,3	7,6	1,56	0,06	1,15	0,83	0,3
26.5 52	4,1	35,9	4,4	33,5	19,5	6,7	1,47	0,18	1,37	0,8	0,28
3.6 52	4,1	40,5	4,5	31	19,2	4,8	1,67	0,18	1,27	0,78	0,2
18.6 52	4,25	44	2,9	33,8	16,4	2,95	1,87	0,12	1,44	0,7	0,12
23.9 52	4,25	34,25	6,35	28,2	19,8	11,4	1,45	0,27	1,2	0,84	0,49
6.10 52	3 9	41	5,7	25,9	20,4	7	1,6	0,22	1,01	0,8	0,27

Date	Prot. tot. %	Alb. %	α_1 %	α_2 %	β %	γ %	Alb. g %	α_1 g %	α_2 g %	β g %	γ g %
					Paul Sj., 3 ans.						
7.4 52	5	43,84	5,56	26,05	17,75	6,8	2,19	0,28	1,3	0,89	0,34
16.4 52	5	47	4,5	23,5	18	7	2,35	0,23	1,17	0,9	0,35
21.4 52	5,2	45	4,9	25,4	15,3	9,4	2,34	0,25	1,34	0,78	0,49
23.4 52	5,2	44,5	6,8	22,9	18	7,8	2,34	0,35	1,19	0,92	0,4
30.4 52	4,6	46	5,9	22	19,7	6,4	2,12	0,27	1,0	0,91	0,3
5.5 52	4,6	46,5	4,8	21,1	18	9,6	2,15	0,23	0,93	0,84	0,45
12.5 52	4,25	50	1,35	23	17,7	7,95	2,12	0,06	0,98	0,75	0,34
19.5 52	4,25	44,3	3,1	24,6	20,8	7,2	2,1	0,13	1,04	0,88	0,30
13.6 52	4,6	47,5	4,2	23,7	16,3	8,3	2,2	0,19	1,09	0,74	0,38
18.8 52	3,9	49	7,1	20,4	15,4	8	1,91	0,28	0,8	0,6	0,31
18.11 52	4,25	43	7,3	22	16,7	11	1,83	0,31	0,93	0,71	0,47
					Georges S., 13 ans.						
28.8 52	4,6	43,5	4,8	19,4	17,6	14,7	2,0	0,22	0,89	0,81	0,68
1.9 52	4	50	4,9	20	14,7	10,3	2	0,2	0,8	0,59	0,41
8.9 52	3,9	38,8	7,05	28,4	17,7	8,05	1,52	0,27	1,1	0,69	0,32
29.9 52	3,9	28,2	6,6	35,2	22,6	7,4	1,1	0,26	1,37	0,88	0,29
6.10 52	3,7	27,6	5,7	32,5	24	10,25	1,02	0,21	1,2	0,89	0,38
27.10 52	3,9	31,6	6,0	32,6	16,7	13,1	1,23	0,24	1,27	0,65	0,51
17.11 52	3,5	30,1	6,6	40,3	17,9	5,1	1,05	0,23	1,41	0,63	0,18
1.12 52	3,7	21,4	3,9	49,4	17,5	7,8	0,79	0,14	1,83	0,65	0,29
					Roger V. N., 1 an et 9 mois.						
31.3 52	3,9	25,6	2,8	37,9	23,9	9,8	0,97	0,18	1,45	0,92	0,38
7.4 52	4,25	37,9	5,8	29,1	20,3	6,9	1,6	0,24	1,2	0,8	0,29
16.6 52	3,9	30,7	4,2	30,3	23,6	11,2	1,2	0,16	1,18	0,92	0,44
6.8 52	5	49,1	4,4	27,1	15	4,4	2,46	0,22	1,35	0,75	0,22
25.8 52	4,3	33,6	6,7	36,7	17,2	5,8	1,44	0,29	1,58	0,74	0,25
13.10 52	3,7	27,6	4,3	35,1	21,5	11,5	1,02	0,16	1,3	0,8	0,42
					Denise V. H., 3 ans.						
14.11 52	5,35	40	4,6	41,6	12,1	1,7	2,14	0,24	2,22	0,65	0,1
					Bernard C., 7 ans.						
3.7 52	3,1	26,4	2,3	44,8	20,6	5,9	0,82	0,07	1,39	0,64	0,18
6.8 52	3,15	21,8	3,6	32,9	33,3	8,3	0,65	0,1	1,03	1,04	0,29
25.8 52	3,1	30,05	7,45	38,55	13,65	10,3	0,93	0,23	1,2	0,43	0,31

TABLEAU 2

Moyenne des résultats électrophorétiques de sérums d'enfants, atteints de néphr[illegible] lipoïdique, groupés d'après l'aspect clinique.

Valeurs relatives:

Etat d'œdème	Nombre d'examens	Protéine totale	Albumine	α_1	α_2	α	β	γ
Sans œdème	28	3,15–6,1 4,34	14,9–47,6 37,2	1,5–7,3 5,5	13–52,2 32,8	16,2–60,7 38,3	13,1–22,2 16,3	2 [illegible] 4,3 8,1
Œdème peu marqué	17	3,1–5 3,86	15,5–49,1 31,7	2,3–6,35 4,4	25,9–56,6 36,38	31,5–60 40,78	15–23,9 19,8	2,9 [illegible] 11,4 7,[illegible]3
Œdème très marqué	16	3,5–4,6 4	21,4–50 35	3,9–11 6	20–49,4 31,24	25–53,3 37,24	13,5–24 18,1	5 [illegible] 14,7 9,1
Œdème généralisé avec ascite	4	3,15–5,35 3,9	18,9–40 24,2	1,1–6,1 3,22	41,6–63,5 52,3	46,1–69,6 55,5	10,1–23,2 17,15	1,4 [illegible] 4,8 3,2

Valeurs absolues:

Etat d'œdème	Nombre d'examens	Protéine totale	Albumine	α_1	α_2	α	β	γ
Sans œdème	28	3,15–6,1 4,34	0,58–2,87 1,68	0,1–0,4 0,23	0,77–1,73 1,39	1,03–2,04 1,62	0,45–1,1 0,72	0,08–0,58 0,36
Œdème peu marqué	17	3,1–5 3,86	0,64–1,87 1,36	0,10–0,27 0,17	1–2,4 1,39	1,13–2,55 1,56	0,4–1,04 0,71	0,12–0,49 0,3
Œdème très marqué	16	3,5–4,6 4	1,1–1,85 1,4	0,14–0,43 0,24	0,8–1,37 1,22	1–1,63 1,46	0,59–0,88 0,72	0,18–0,68 0,38
Œdème généralisé avec ascite	4	3,15–5,35 3,9	0,5–14 1,03	0,04–0,24 0,14	2–2,22 2,05	2,04–2,46 2,2	0,32–0,9 0,69	0,05–0,19 0,13

TABLEAU 3

Moyenne des résultats électrophorétiques de sérums d'enfants, atteints de néphrose lipoïdique, groupés d'après les protéines totales.

Protéine totale	Moyenne des protéines totales	Nombre d'examens	Albumine	α_1	α_2	α	β	γ
3–3,99	3,1–3,9 3,7	30	14,9–49 29,6	1,1–11 4,91	20,4–63,5 38,94	27,5–69,6 43,85	10,1–33,3 18,68	1,4–13,1 7,86
4–4,99	4–4,6 4,33	24	15,5–50 39,10	1,3–8 5,07	18–56,6 29,7	24–60 34,8	14,5–21,1 17,8	2–14,7 7,81
5–5,99	4–5,75 5,29	13	26,1–56 42,02	2–7,5 5,43	19,8–41,6 28,13	24,7–46,1 33,56	11,7–22,2 15,88	4,3–12,2 8,08
6–6,99	6,1	1	47,1	4	23,2	27,2	20,7	5

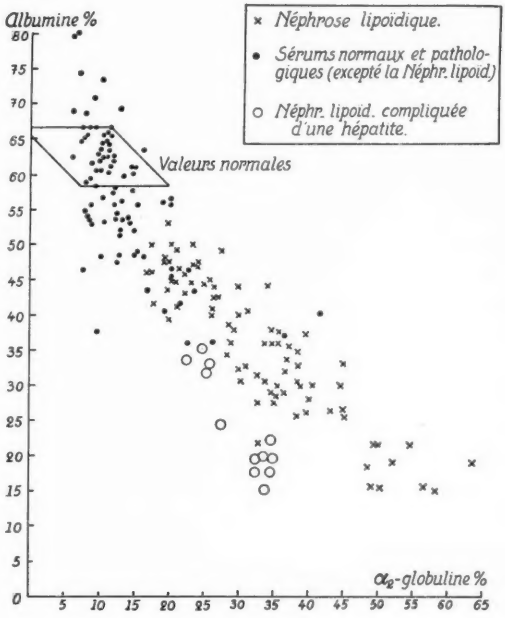

Fig. 1.

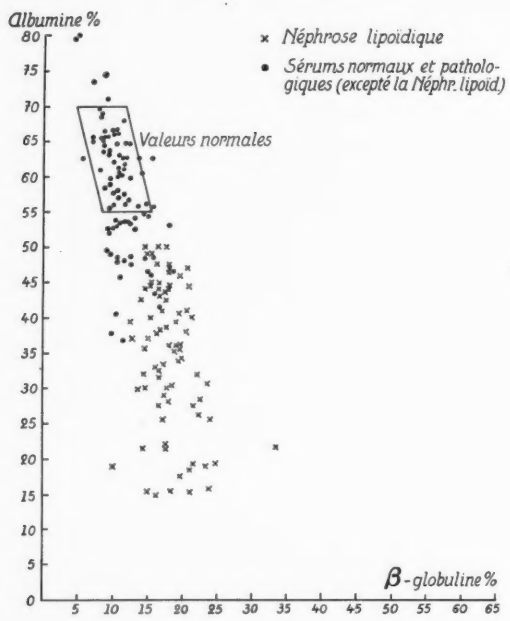

Fig. 2.

Il existe aussi une certaine relation entre la globuline β et l'albumine, mais elle n'est pas si prononcée qu'entre la globuline α_2 et l'albumine (Fig. 2).

Nous n'avons pas pu constater de relation entre la globuline γ et l'albumine. Dans la plupart des dysprotéinémies un abaissement de l'albumine va de pair avec une augmentation absolue et relative de la globuline γ (hépatite, cirrhose, myélome etc.). La néphrose lipoïdique occupe une place très spéciale: on constate à la fois un taux très bas d'albumine et de globuline γ.

Analysé sous un autre aspect, il existe un rapport entre la globuline α et la globuline γ.

RIVA a étudié pour différents états pathologiques la corrélation possible des différentes fractions des globulines entre elles. En déterminant la relation des variations du taux des globulines α et γ par rapport aux globulines totales (et non plus par rapport aux protéines totales), dans plusieurs dysprotéinémies, RIVA parvient à mettre en évidence une corrélation frappante entre les globulines α et γ et ceci pour différents groupes de maladies.

Nous avons recherché cette relation avec des sérums d'enfants normaux et d'enfants atteints de néphrose lipoïdique ou d'autres affections comme la néphrite, l'hépatite épidémique, la maladie coeliaque etc. (Fig. 3). Nous avons

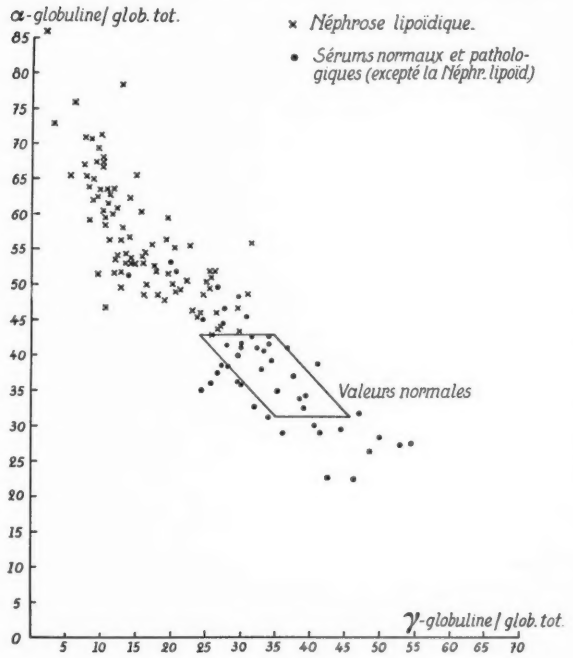

Fig. 3.

pu confirmer les constatations de RIVA, que la valeur du rapport entre globulines α et γ est caractéristique d'un état pathologique déterminé.

Le rapport obtenu par un taux très élevé de globuline α et un taux très bas de globuline γ situe les valeurs trouvées dans la néphrose lipoïdique au sommet de la droite. A l'autre extrémité se situent les valeurs du rhumatisme articulaire et des états de septicémies avec un taux très élevé de globuline γ et un taux très bas de globuline α.

Globulines β Lipides

Nous avons recherché le rapport existant entre les globulines β et les lipides totaux, en mettant les valeurs absolues des globulines β en g% en abscisses, et les valeurs des lipides totaux en g% en ordonnées.

Il n'existe pas de corrélation entre les valeurs absolues des globulines β, déterminées avec l'appareil Elphor, et les lipides totaux (Fig. 4). Cependant, sur 25 sérums étudiés avec l'appareil de Kern, il y a une relation nette entre les valeurs absolues des globulines β et les lipides totaux (Fig. 5). L'un

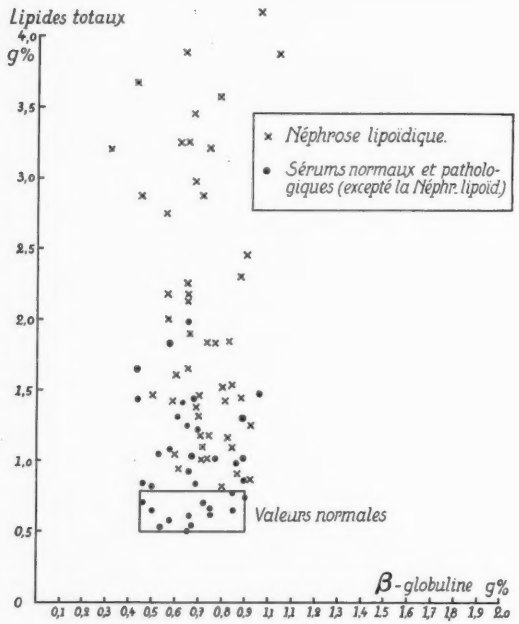

Fig. 4.

des avantages de l'appareil Elphor est que les résultats ne sont pas faussés par la présence des lipides.

Depuis les travaux initiaux de LONGSWORTH et MACINNES, on a d'ailleurs pu montrer que les valeurs absolues et relatives des globulines β dans la néphrose lipoïdique sont beaucoup moins élevées, lorsqu'on a au préalable fait une extraction des lipides.

Dans une étude sur la répartition des lipides dans les lipoprotéines α et β EDER e. a. donnent les résultats suivants pour un sujet normal: 29 % de la cholestérine totale se retrouve dans la lipoprotéine α, 64 % dans la lipoprotéine β. Chez un malade atteint de néphrose lipoïdique, ils trouvent 92 % de la cholestérine dans la lipoprotéine β et seulement 5 % dans la lipoprotéine α.

C'est en employant une méthode optique comme celle de Kern, où l'indice de réfraction est fortement influencé par les lipides, qu'on peut constater un certain rapport entre les variations des lipides et de la globuline β.

KUNKEL et AHRENS ont constaté que dans d'autres états pathologiques que la néphrose lipoïdique, la méthode optique d'après TISELIUS donne des résultats pour la globuline β qui sont fortement influencés par la présence des lipides. Ils trouvent le même rapport linéaire entre les valeurs relatives de la globuline

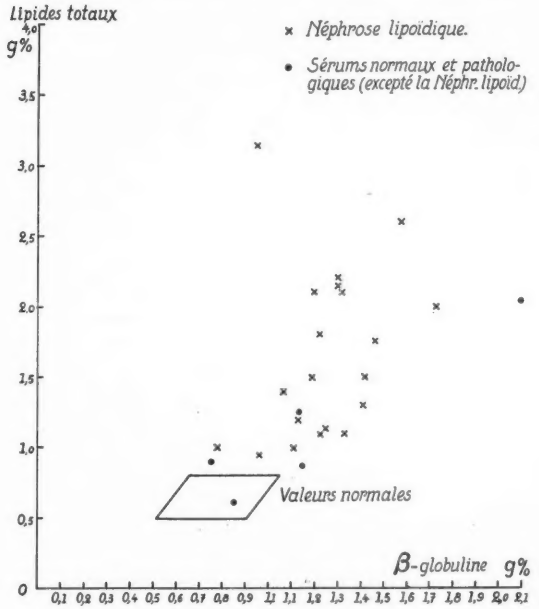

Fig. 5.

β et les lipides totaux que nous avons constaté avec l'appareil de Kern pour les valeurs absolues de la globuline β et les lipides totaux.

On ne retrouve plus cette relation en employant l'appareil Elphor, les chiffres n'étant pas obtenus par une méthode réfractométrique.

Conclusion

L'examen électrophorétique de sérums d'enfants atteints de néphrose lipoïdique nous montre une répartition typique des différentes fractions protéiques: diminution considérable de l'albumine (valeurs relatives et absolues), valeurs relatives un peu au-dessus de la normale et valeurs absolues diminuées pour la globuline α_1; augmentation constante de l'α_2 (valeurs relatives et absolues); augmentation des valeurs relatives de la globuline β, les valeurs absolues étant normales; diminution des valeurs relatives de la globuline γ, diminution considérable des valeurs absolues.

Pour la globuline β les résultats varient selon la méthode employée. Ainsi nous avons trouvé avec la méthode de Kern des moyennes trop élevées pour la globuline β l'examen étant faussé par les lipides (9 cas de néphrose lipoïdique, 10 sérums examinés — moyenne pour la globuline β 24,7 % ou 1,16 g %).

Les valeurs relatives et absolues des différentes fractions varient de malade à malade. Il n'y a pas de concordance nette entre ces variations et la présence ou l'absence d'œdème dans la période évolutive. On ne peut dégager un rapport constant si l'on compare les valeurs relatives des différentes fractions au taux des protéines totales. Dans la néphrose lipoïdique ce n'est pas l'hypoalbuminémie seule qui est pathognomonique, mais plutôt l'augmentation considérable de la globuline α_2 et la diminution de la globuline γ.

Il existe un rapport linéaire entre les valeurs relatives de l'albumine et de la globuline α_2 par rapport aux protéines totales, ainsi qu'entre la globuline α et la globuline γ par rapport aux globulines totales. Analysée sous cet aspect la néphrose lipoïdique se caractérise nettement des autres états de dysprotéinémies.

Les données électrophorétiques sont pathognomoniques pour la néphrose lipoïdique, mais n'apportent aucun élément de pronostic.

Résumé

Les sérums de 8 enfants atteints de néphrose lipoïdique pure et d'un enfant présentant une néphrose lipoïdique compliquée d'atteinte rénale ont été examinés par la micro-électrophorèse sur papier (Procédé de GRASSMANN et HANNIG: appareil Elphor-H). Les avantages et les désavantages de cette microméthode, comparés à ceux de la macro-électrophorèse d'après TISELIUS sont discutés. Chez tous les malades, atteints de néphrose lipoïdique, le taux de l'albumine est très bas; celui de la globuline α_2 toujours très élevé; la valeur absolue de la globuline γ est toujours très basse. Les valeurs relatives de la globuline α_1 sont un peu au-dessus de la normale. Les résultats obtenus pour la globuline β dépendent de la méthode employée; les valeurs sont plus élevées avec les méthodes réfractométriques qu'avec l'appareil Elphor. Avec la méthode optique, l'indice de réfraction de la globuline β est fortement influencé par les lipides. Les pourcentages et les valeurs absolues des différentes fractions varient sensiblement de malade à malade. Il n'y a pas de concordance nette entre la répartition des différentes protéines dans la néphrose lipoïdique et les états d'œdème. On ne peut trouver de relation entre les valeurs relatives des fractions protéiques et le taux des protéines totales.

The results of a study on the serum in infants suffering from lipoid nephrosis by means of paper microelectrophoresis.

Serum samples from 9 children with lipoid nephrosis, in one case complicated by a renal disease, and from 17 normal children have been analysed by paper microelectrophoresis (procedure of Grassmann and Hannig, apparatus Elphor-H). The distribution of the serum protein fractions showed in all cases of lipoid nephrosis a very low concentration of albumin and an important increase of the α_2-globulin. There is always a decrease of the absolute concentration of γ-globulin. The percentage distribution of the α_1-globulin is somewhat below the normal range. The results obtained for the β-globulin fractions depend of the methods used: they are much higher with an optic apparatus than with the Elphor apparatus. The percentage distribution and the

absolute "values" of the different fractions vary considerably from one patient to the other. There is only a slight correlation between the distribution of the different serum protein fractions and the clinical evolution of the edemas. There is no correlation at all between the total protein level and the percentage distribution of the different serum fractions. There is a very apparent correlation between the percentage distribution of the α_2-globulin and the percentages of the albumin. This relationship exists but is less pronounced between the β-globulins and the albumin. It does not exist between the α_1- and γ-globulins and the albumin.

Eine Studie über das Serum von Kindern mit Lipoidnephrose durch die Papier-Mikroelektrophorese analysiert.

Das Serum von 9 Kindern mit Lipoidnephrose, die bei einem durch eine Nierenkrankheit kompliziert war, und 17 normalen Kindern wurde durch die Papier-Mikroelektrophorese analysiert (nach der Methode von GRASSMANN und HANNIG, Apparat Elphor-H). Die Verteilung der Serumproteinfraktionen zeigte in allen Fällen von Lipoidnephrose eine sehr geringe Albuminkonzentration und eine bedeutende Vermehrung der α_2-Globuline. Es besteht immer eine Verminderung der absoluten γ-Globulin-Konzentration. Die prozentuelle Verteilung der α_1-Globuline liegt ein wenig unter den normalen Grenzen. Die Werte, die für die β-Globulinfraktionen gefunden wurden, hängen von der angewandten Methode ab. Bei der Bestimmung mit einem optischen Apparat liegen sie höher als mit dem Elphor-Apparat. Die prozentuelle Verteilung und die absoluten Werte der verschiedenen Fraktionen variieren bedeutend von Patient zu Patient. Es besteht nur eine geringe Beziehung zwischen der Verteilung der verschiedenen Serumproteinfraktionen und der klinischen Entstehung von Ödemen. Es besteht keine Beziehung zwischen dem Totalproteinwert und der prozentuellen Verteilung der verschiedenen Serumfraktionen. Eine sehr wahrscheinliche Beziehung besteht zwischen der prozentuellen Verteilung der α_2-Globuline und der Albumine. Diese Beziehung besteht in weniger ausgesprochenem Maße zwischen β-Globulinen und Albumin. Sie besteht nicht zwischen den α_1- und γ-Globulinen und dem Albumin.

Resultados de un estudio de sueros de niños con nefrosis lipoídica por la microelectroforesis sobre papel.

Sueros de 9 niños con nefrosis lipoídica pura y de un niño con nefrosis lipoídica con toque renal, fueron examinados por la microelectroforesis sobre papel (procedimiento de Grassmann y Hannig: aparato Elphor H). Las ventajas y desventajas de este micrométodo son discutidas. En los enfermos con nefrosis lipoídica los índices de albúmina son bajos, la globulina α_2 muy elevada, el valor absoluto de la globulina γ muy bajo. Los valores relativos de la globulina α_1 están un poco sobre lo normal. Los resultados obtenidos para la globulina β dependen de los métodos empleados, siendo los valores mas elevados con los métodos refractométricos que con el aparato de Elphor. Los porcentajes y valores absolutos de las diferentes fracciones varían sensiblemente de enfermo en enfermo. No hay correspondencia entre la repartición de las diferentes proteinas en la nefrosis lipoídica y los estados de edema. No hay correlación entre los valores relativos de las fracciones proteinicas y el índice de las proteinas totales. Hay una relación evidente entre la globulina α_2 y la albúmina. Esta relación existe pero es menos pronunciada entre las globulinas β y la albúmina. No existe entre las globulinas α_1 y γ y la albúmina.

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Biophysical Studies on Bone Tissue

III. Osteopetrosis (Marble Bone Disease)

by B. ENGFELDT, A. ENGSTRÖM and R. ZETTERSTRÖM

Osteopetrosis is characterized by a diffuse severe osteosclerosis of the whole skeleton. Due to the increase of the calcified tissue there is a reduced amount of hematopoietic marrow leading to severe anemia. Despite the osteosclerosis the bones are very fragile.

The cause of the disease is unknown. The disease is usually congenital and there is a strong familial tendency. Investigations on the calcium metabolism have not given any evidence that the cause of the increased mineralization of the skeleton is due to a primary disturbance of the mineral metabolism (2). The most widely accepted theory is that the primary cause of the osteosclerosis is a reduction of osteoclastic activity and that the retarded resorption results in an occlusion of the marrow cavity with a tissue consisting of calcified cartilage and immature bone (8).

In the present paper results from an investigation of two cases of osteopetrosis will be communicated. In order to obtain information concerning the nature of the disease different biophysical methods have been applied. The nature of the mineral salts and their crystalline structure have been studied by wide angle X-ray diffraction. The distribution of mineral salts in bone tissue has been studied by X-ray micro absorption measurements and the arrangement of the collagen in decalcified sections with polarized light.

Material

The specimens we have investigated are from two patients suffering from osteopetrosis. In both instances the disease was congenital and associated with a strong hereditary tendency.

Case 1. The infant died at the age of five months. The case history has been described by SUNDAHL (7). At autopsy the bones were severely osteosclerotic, the marrow cavity in the long bones being totally filled with calcified tissue. We are indebted to Prof. SUNDAHL, Bergen, Norway, for sending the autopsy specimen.

Case 2. This individual, who is still alive, was at biopsy (1952) three years old. At this age there was severe osteosclerosis with anemia and extramedullary hematopoiesis. The case history will be described in full detail by ENELL and PEHRSON. We are indebted to Dr. KOSTMAN, Boden, Sweden, for sending the biopsy specimen.

Methods

Microradiography.—For this investigation 50–150 micra thick ground sections of the bone specimen were used. For comparison material, sections of the same thickness from the same bones were taken from autopsy material of children of the same age who showed no signs of bone disease. An ordinary diffraction unit with a copper target X-ray tube served as source of X-rays. The X-rays were generated at 25–40 KV. Decalcified sections did not show any absorption at all indicating that with the wavelength used the image produced was due only to the content of mineral salts. Maximum resolution plates were used to record the image. Enlarged pictures of the microradiograms were obtained by photomicrography.

Polarized light.—The ground bone sections were completely decalcified in trichloroacetic acid and mounted in saline and photographed in polarized light obtained from crossed Nichol prisms.

X-ray diffraction was performed on 50–100 micra thick ground cross or longitudinal sections from the compact bone of the tibia. A flat cassette camera with a sample at film distance of 2.5 cm was used with copper Kα-radiation filtered in Ni. Powdered specimens were investigated with the aid of a cylindrical camera with 57.4 mm diameter.

Results

Microradiography. — Figure 1 A shows an enlarged microradiogram of a cross section of the shaft of a tibia of a child who was five months old at death and showed no signs of bone disease. It can be clearly seen that different parts of the bone have a different absorption of X-rays. The osteocytes, having no mineral deposit, appear as round or spindle-shaped areas transparent to X-rays. Different Haversian systems have a different content of mineral salts. The Haversian systems usually are less calcified than the old bone surrounding them.

The microradiographic picture of normal bone has been described by AMPRINO and ENGSTRÖM (1). They have shown that different Haversian systems (osteons) have different absorptions of the X-rays. They were able to show also that the degree of absorption varies according to variations in the relative content of mineral salts. Thus, the distribution of minerals is not uniform in the bone tissue, some units having a high content of mineral salts, while in other parts the concentration is markedly lower. Since the uptake of radioactive phosphate is most rapid in Haversian systems having a low content of mineral salts (3), and since the resorption cavities under normal conditions are surrounded by bone tissue having a high content of mineral

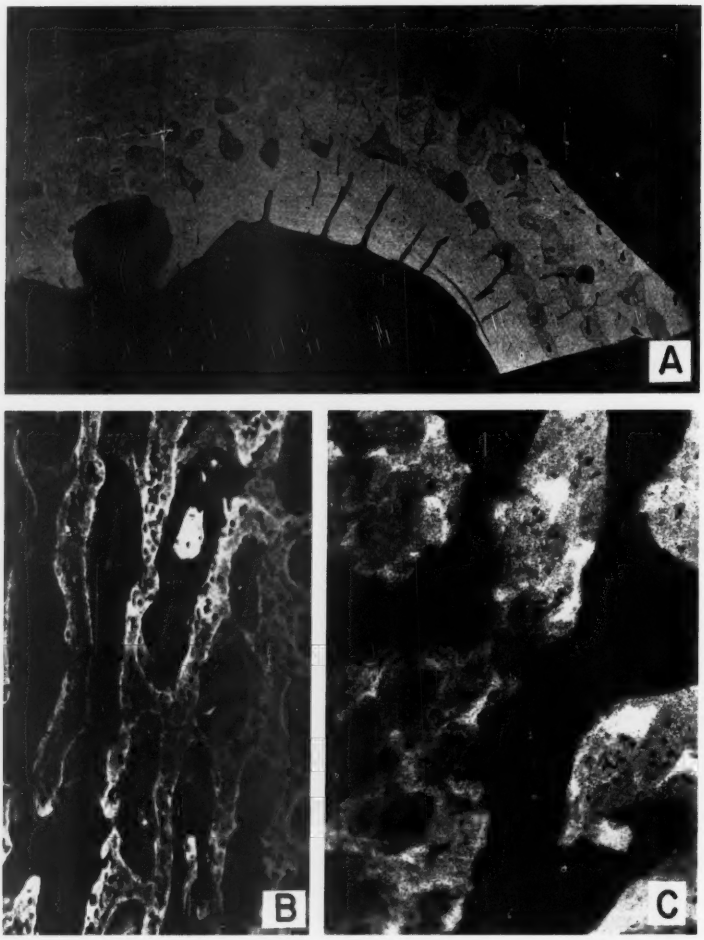

Fig. 1. *A*. Microradiogram of a *ca.* 150 micra thick cross section of a part of the diaphysis of the tibia from a 5 months old child with no known bone disease. Low magnification. The Haversian systems (osteons) are less calcified than the surrounding bone tissue. The content of calcium salts varies from one osteon to another in such a way that a younger osteon is less calcified than an older one.
B. Microradiogram of a cross section of the inner part of the "compact" bone in the diaphysis of the tibia from a case of osteopetrosis (infant, 5 months old). High magnification.
C. Microradiogram of the marrow bone from the same case as in 1 *B*. High magnification.

salts, systems with a low content of mineral salts must be young systems. Thus, during the calcification of bone there is, at the beginning, no full deposition of mineral salts in the structures awaiting calcification. The content of mineral salts gradually increases. In all bone structures there is a contin-

areas rebuilding, resorption cavities are formed and are then filled with newly Haversian systems. In growing animals the reconstruction of the bone tissue is more pronounced than in the adult ones. For that reason the number of Haversian systems having a low content of mineral salts is greater in the young animals than in the adults.

Fig. 1 B and C and Fig. 2 are enlargements of microradiograms of a cross section from the tibia in osteopetrosis (Case 1). The bone filling up the marrow is shown in Fig. 1 C. The microradiogram shows that the structure and the distribution of mineral salts of this calcified tissue are quite different from what can be seen in the normal bone tissue. Throughout the whole calcified tissue there is an enormous variation in the content of mineral salts in different parts of the trabeculae. Areas transparent to X-rays, i.e. areas containing osteocytes, are arranged in groups, such cell-containing structures having a very low degree of calcification. These areas are surrounded by a sharp boundary of acellular tissue which shows a high content of mineral salts. The proportion between highly calcified tissue and cell-containing, calcium-poor areas varies in different trabeculae. There is a strong resemblance between the mineral salt distribution of these marrow bones and the calcified tissue formed when experimentally induced hypertrophic rachitic cartilage is undergoing calcification following treatment with vitamin D (unpublished). There is no doubt that calcification processes are going on in such areas. In other parts of the trabeculae where the calcium content of the periphery of the trabeculae is very high and the boundary is very sharp there must be a resorption of the calcified tissue. It also seems that some of the poorly calcified parts of the trabeculae are undergoing resorption. In occasional areas resorption cavities are formed within the trabeculae. Some of these cavities are filled with a bone structure resembling Haversian systems.

Thus, in this abnormal marrow bone there are indications of a very high calcification activity as well as of an intense resorption. No replacement of this tissue by normal spongy bone tissue is seen. The type of mineral deposition is the same as that seen when there is a calcification of hypertrophic rachitic cartilage. From histological investigations this tissue has been described as densely calcified bone trabeculae lined with osteoblasts. The trabeculae have been considered as necrotic bone since they contain many empty osteocytes, irregular calcified tissue and loose connective tissue (8). From the microradiographic examination it is, however, quite clear that this bone structure cannot be necrotic.

It has long been known that in osteopetrosis the bone tissue filling the marrow cavity is quite different from that of subperiosteal bone. This part of the bone in structure resembles more the normal bone tissue although there are no distinct Haversian systems, canaliculi or laminations (2).

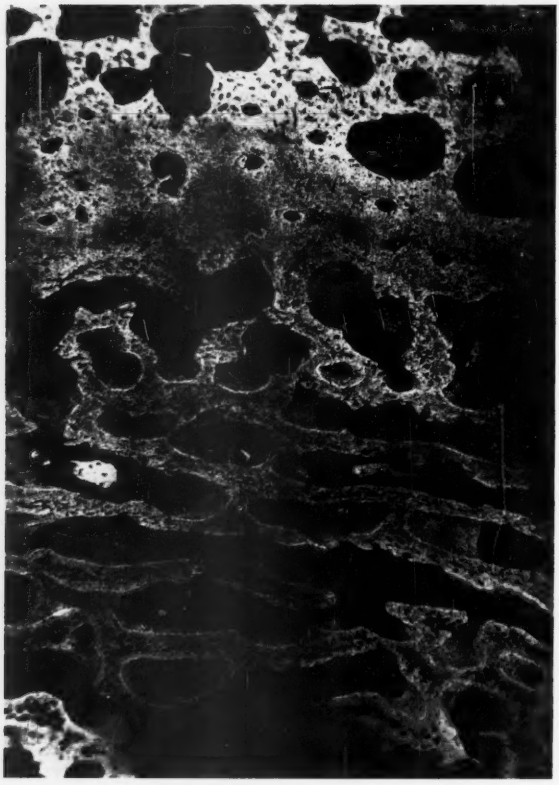

Fig. 2. Cross section (100 micra) of the outer part of the tibia from a case of osteopetrosis microphotographed with 25 KV X-rays showing the distribution of mineral salts.

Fig. 2 shows a microradiogram of a cross section of the bone surrounding the marrow tissue in the tibia of case 1. In the center of this tissue there is a relatively thin outline of almost normal bone tissue having Haversian systems made up of regularly arranged osteocytes and having Haversian canals of ordinary size. This zone, which corresponds to the compact bone tissue of the shaft, is made up of a tissue which is almost normal in structure and mineral distribution, but which varies in thickness in different cross sections of the tibia.

On both sides of this area development is abnormal, but different from the type of the calcified tissue that fills the marrow cavity. In the inner zone there are numerous large and irregular resorption cavities, in parts of which calcification is in progress, while in other parts there is an intense resorption

of the bone trabeculae. In all parts of the cavities there is a sharp boundary between calcified and non-calcified tissue. The intense rebuilding of this part of the skeleton leads to the formation of a bone structure made up of irregularly arranged areas of high and low calcification. Between areas of differing mineral salt content, hence differing X-ray absorption, there is a relatively broad transitional zone which is highly calcified. Such a broad zone can never be seen in normal bone, where the limit between structures having differing mineral contents is always clear. A clear distinction between different zones was also apparent in the more normal parts of this specimen. The arrangement of the small X-ray transparent areas within the bone trabeculae, i.e. the places for the bone cells, is quite irregular and their width is much greater than in normal adult bone. The bone trabeculae are coarsely fibrillar and immature in appearance, thus resembling the bone trabeculae formed in osteogenesis imperfecta (unpublished). In the outermost part of the bone resorption cavities are very much larger than in its inner parts and occur frequently.

The limit between the two definitively different types of calcified tissue occurring in the skeleton in osteopetrosis is very sharp and there are no islets of marrow bone in the outer zone of the bone. This fact seems to indicate that the two zones are of different origin and suggests that the marrow bone is formed by endochondral and the other type by periosteal bone formation. Both types are, however, quite abnormal.

Microradiograms of cross sections from the ventral part of a rib from case 2 showed that the structure and mineral distribution in the whole specimen is the same as in the calcified tissue filling up the marrow cavity of a long bone from case 1. In this spongy bone there is no real osteophyte.

Polarized light. — The regular arrangement of the collagen fibres in the normal compact bone is demonstrated in Fig. 3 A. As can be seen from Fig. 3 B and 3 C, which are from a cross section from the tibia of case 1, the arrangement of collagen fibres is quite abnormal in osteopetrosis. Instead of concentric layers of collagen fibres there are short broad bundles, which are either irregularly arranged or show a tendency to the same orientation as in Haversian systems. In some parts of the bone the collagen bundles are mainly orientated circularly around the bone axis. Numerous very thin stripes running perpendicularly between the broad main bundles can also be seen (Fig. 3 C). In Figures 3 B and 3 C the collagen content is relatively high. In other sections, however, the collagen content is much reduced, and in polarized light only few spots of collagen can be seen although the undecalcified section has a high content of mineral salts. The highly abnormal arrangement of the collagen fibres seems to give a satisfactory explanation for the fragility of the bones in osteopetrosis.

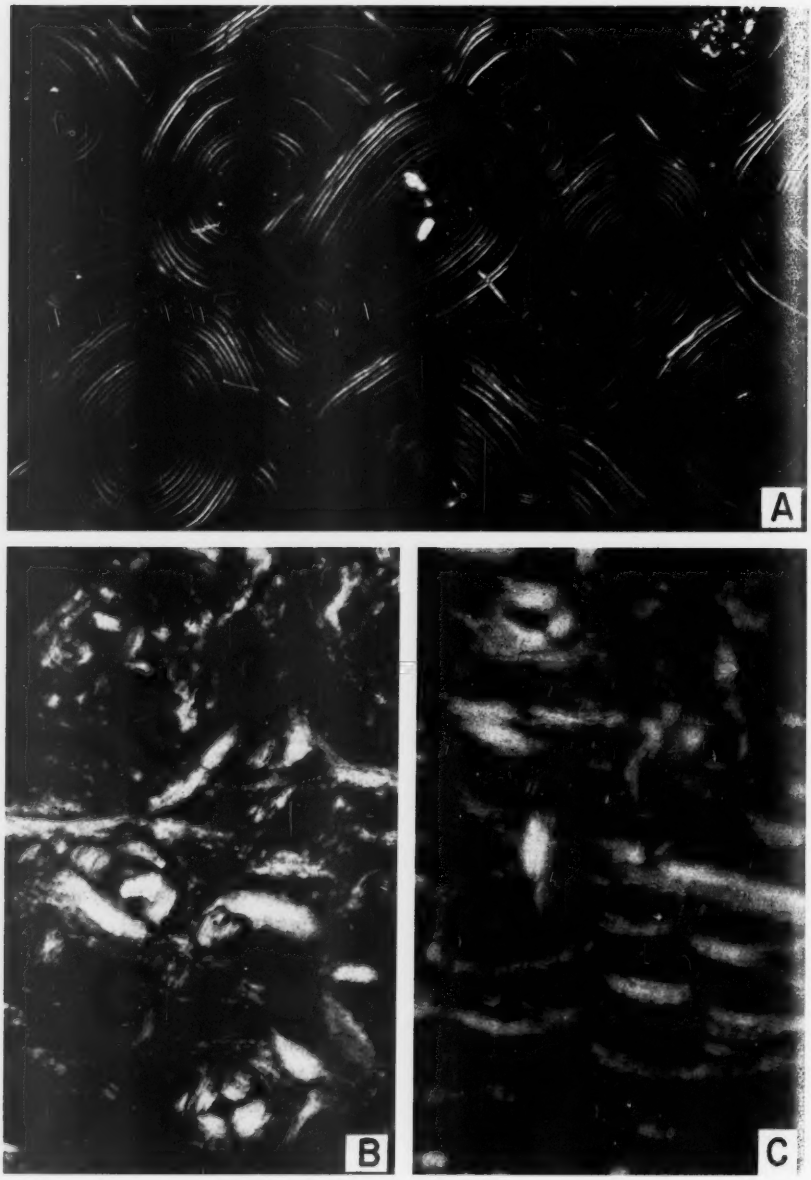

Fig. 3. Photomicrographs in polarized light of decalcified bone sections. These pictures sho the distribution of collagen.

A. Normal bone, cross section. Note the regular arrangement of bundles of collagen fibres i the Haversian systems.

B. and *C*. Cross sections of the diaphysis from a case of osteopetrosis (case 1) showing th inner and outer zones of the bone respectively.

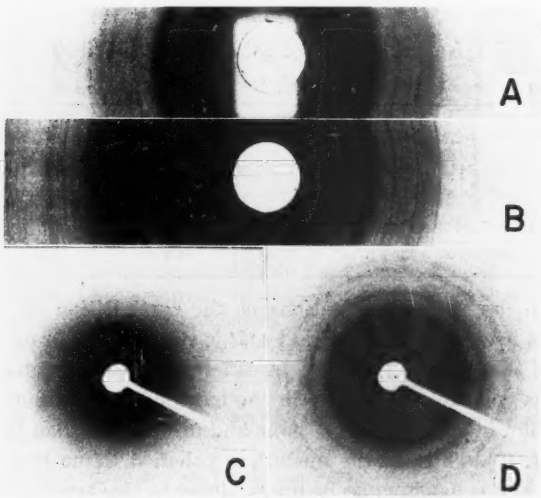

Fig. 4. *A*. X-ray powder diagram of marrow bone from a case of osteopetrosis.
B. The same as in *A* but heated. Note the sharpening of the diffraction lines indicating growth of the crystallites.
C. Flat film X-ray diagram of a longitudinal section of the osteophyte. Mostly organic pattern indicating low calcification.
D. Longitudinal section from the medullary bone in one flat film camera. No orientation (arcing of the rings) can be seen.

The nature of the bone salts in osteopetrosis. — The wide angle *X-ray diffraction diagram* of osteopetrotic bone could not be distinguished from that of normal bone either in the osteophyte or in the calcified tissue filling the bone marrow cavity. The crystalline structure, as far as can be judged from this method, is thus the same as in normal bone. The mineral salt is probably hydroxylapatite in a hexagonal lattice with the c-axis = 6.87 Å, and the a-axis = 9.41 Å which agrees with the findings of other investigations on the bone salt (6, 4). Figures 4 A and B show powder diagrams from the marrow bone. In Figure 4 B the same powder as in Figure 4 A was heated, resulting in a sharpening of the diffraction lines. Figure 4 A shows that the diffraction lines are rather diffuse indicating that the crystallites are small. Thus, the exchangeability of the ions forming the mineral salts in the marrow bone must be high, cf. (10). As in normal bone, however, the crystallites grow on heating (Figure 4 B). Figure 4 C and D shows flat film diagrams from thin longitudinal sections of the osteophyte and marrow bone. In the osteophyte there are mostly organic patterns, i.e. collagen patterns, indicating very low mineralization. In the diagram from the marrow bone no orientation of the mineral salts can be seen.

TABLE 1

Ash content and the composition of ash in the tibia from a case of osteopetrosis.

	Ash content in fat-free bone per cent	Molecular ratio Ca:P
Medullary bone	70	1.59
Osteophyte bone.	59	1.57

From macrochemical investigations of the composition of the bone ash, cf. Table 1, it is clear that the ratio Ca:P is the same as in normal bone, cf. (5), and that the ratio is the same in different structures of the bone. The ash content is considerably higher in the calcified tissue filling up the marrow cavity than in the osteophyte bone and also higher than in normal compact bone, cf. (9). Thus, in the medullary calcified tissue the ratio between mineral salts and collagen must be much higher in this type of calcified tissue than in the subperiosteal bone. The low collagen content in some parts of the medullary bone has also been demonstrated by the microphotograms in polarized light.

Discussion

According to the features characteristic of osteopetrosis demonstrated in this investigation the cause of the disease cannot be solely an inhibition of osteoclastic resorption. Typical resorption areas can be shown to occur in the different types of bone tissue resulting from this disease. In the osteophyte the resorption processes seem to be as active as in chronic hyperparathyroidism. Nor can the cause of the disease be an abnormality of the calcium metabolism. The different types of calcified tissue resulting from this disease are the same as can be seen during the development of normal bone. The medullary bone is the same as in the metaphysis of normal growing bone, and the osteophyte is the same as primitive bone previous to rebuilding.

The mechanism, involved in the replacement of the immature bone tissue by adult bone, is disturbed in this disease in such a way that this bone tissue cannot be substituted for by normal adult bone in the cortical layer and by haematopoietic tissue in the marrow cavity. The cause of this abnormality is to be found in some functional anomaly of the bone-forming cells.

Summary

In two cases with osteopetrosis the bone tissue has been studied by biophysical methods. The ultrastructure has been studied with X-ray diffraction and the distribution of mineral salts with microradiography: the molecular structure is the same as in normal bone. The distribution of the mineral salts is quite different from normal

bones, but shows characteristic patterns. The arrangement of collagen has been investigated in polarized light, the arrangement being quite irregular, a fact which seems to give a satisfactory explanation for the fragility of the bones. The cause of the disease is to be found in some functional anomaly of the bone-forming cells.

Études biophysiques du tissu osseux. III: L'Ostéopétrose (Maladie des os de marbre).

Des études du tissu osseux dans deux cas d'ostéopétrose ont été réalisées par des méthodes biophysiques. L'ultrastructure a été étudiée par la diffraction des rayons X, et la distribution des sels minéraux par la microradiographie. La structure moléculaire reste la même que dans l'os normal. Par contre, la distribution des sels minéraux est complétement différente de celle de l'os normal, et montre des dispositions très caractéristiques. La disposition du collagène a été recherchée par la lumière polarisée; elle est complètement irrégulière, ce qui semble expliquer de façon satisfaisante la fragilité osseuse. La raison de la maladie doit résider dans une anomalie fonctionnelle des ostéocytes.

Biophysikalische Studien des Knochengewebes. III. Osteopetrosis (Marmorknochenkrankheit).

Bei zwei Fällen von Osteopetrosis wurde das Knochengewebe mit biophysikalischen Methoden untersucht. Die Ultrastruktur wurde durch Röntgenstrahlendiffraktion studiert und die Verteilung der Mineralsalze mit Hilfe der Mikroradiographie. Die Molekularstruktur war dieselbe wie in normalen Knochen. Die Verteilung der Mineralsalze ist von der in normalen Knochen ganz verschieden, zeigt aber charakteristische Strukturen. Die Anordnung des Kollagens wurde im polarisierten Licht untersucht und zeigte eine unregelmäßige Anordnung. Diese Tatsache gibt eine ausreichende Erklärung für die Zerbrechlichkeit der Knochen. Die Ursache der Krankheit scheint in einer funktionellen Anomalie der knochenbildenden Zellen zu liegen.

Estudios biofísicos en el tejido óseo. III. Osteopetrosis (Enfermedad Marmórea).

En dos casos de osteopetrosis el tejido óseo ha sido estudiado por métodos biológicos. La ultraestructura ha sido estudiada por difracción de rayos X y la distribución de sales minerales por microradiografía: la estructura de la molécula es la misma de un hueso normal. La distribución de sales minerales es bastante distinta a la de un hueso normal, pero tiene caracteres típicos. La distribución de colágeno ha sido investigado con luz polarizada, siendo muy irregular, lo que parece dar una explicación satisfactoria para la fragilidad de los huesos. La causa de esta enfermedad parece ser una anomalía funcional de las células formadoras de hueso.

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Persistence of Tuberculin Sensitivity in BCG Vaccinated Persons Isolated in Institutions

by OLE WASZ-HÖCKERT and MÄRTA DONNER

The various types of vaccination always leave pediatricians face to face with the same ever-topical problem—how long does the vaccine protect the inoculated? With BCG vaccination the answer is not easy to find. Although we know that immunity and tuberculin-positivity are not overlapping concepts we must in practice state that the protection lasts as long as the BCG vaccinated subject remains tuberculin-positive. But it is also difficult to know how long tuberculin-positivity persists after vaccination. Almost all the published investigations of this problem have one great failing, viz. that the milieu of the BCG vaccinated subjects has to some extent been one in which the risk of tuberculous infection cannot be excluded with certainty. In other words, we do not know for certain what percentage of an investigation material has later not only been subjected to deliberate, bovine BCG inoculation but has also been exposed to natural, virulent, human TB infection.

The present report is based on an investigation initiated in 1949. At the time of writing it covers a total of 217 persons who have lived during the investigation in a milieu completely isolated from tuberculous infection, viz. in closed institutions for mental defectives where both the patients and the staff were under observation of tuberculosis. Although the investigation period, 3 $^1/_2$ years, is too short, we wish to give an interim report on our results to date.

Table 1 gives a survey of the literature on the subject. It indicates the author, year of publication, place of investigation, and composition of material classified according to the risk of infection in the milieu in question. The investigations are relatively few in number and mainly from the Scandinavian countries. Six of them have been carried out in part among persons living in "tuberculosis-free" milieus. They have been concerned with persons from homes where no risk of infection is known to exist, but the possibility of infection at work or in school could not be excluded. Only RINVIK's ma-

TABLE 1

Earlier investigations into the duration of tuberculin allergy after BCG vaccination.

Author	Year of publication	Place Material	Exposure to tuberculosis				
			Total examined	Tuberc. milieu	Uncertain exposure	Tub.-free milieu	No mention
ANDERSSON & BELFRAGE	1939	Gothenburg School children & adults	905	397	207	301	—
DAHL, HERTZBERG, REFSUM	1941	Oslo School children & adults	2,354	—	—	—	2,354
RINVIK	1944	Oslo Infants	30	—	—	30	—
RYDÉN	1946	Stockholm Infants	371	—	—	—	371
TÖRNELL	1947	Borås, Sweden Children & adults	707	108	31	568	—
BLUHM	1948	Stockholm 0—35 years	400	74	—	326	—
WASZ-HÖCKERT	1948	Stockholm 0—14 years	1,702	108	188	1,406	—
GEDDE-DAHL	1951	Oslo Adults	817	—	—	—	817
ENELL	1952	Stockholm School children	630	—	—	—	630
EDWARDS, PALMER, MAGNUS	1953	Denmark School children	658	—	—	658	—

terial derives from a closed institution absolutely excluding the risk of tuberculous infection. In all these investigations vaccination had been effected intracutaneously. In addition, one investigation concerns a perorally vaccinated material (PARK, KERESZTURI and MISCHULOW).

Material

The investigations were made in the following 6 institutions for mental deficiency; the Rinne Home of the Helsingfors Deaconess Hospital in Helsingfors and Esbo, the Home Mission Society's institutions in Kuopio Rural Commune, Rautalampi and Pieksämäki, and the City of Helsingfors institution at Nurmijärvi.

Number of inmates tuberculin tested	Tuberculin-positive	Tuberculin-negative	Previously BCG vaccinated
565	226	269	70

TABLE 2

Age distribution.

Age, years	0—2	3—6	7—10	11—13	14—16	17—23	24—31	Total
Number	2	24	62	48	25	53	13	227
Percentage.	0.9	10.6	27.3	21.2	11.0	23.3	5.7	100

Of these 269 tuberculin-negative (to 1.0 mg Mantoux) 227 were BCG vaccinated. Their age distribution can be seen from Table 2.

All 227 have been taken care of at the above closed institutions for mental deficiency in all its various forms. Forty-nine or 21.5 per cent of them were mongoloids. Although the tuberculin reactions of these mongoloids differ considerably from those of the material as a whole (DONNER, not yet published) the entire 227 patients have been included in the material as the results do not statistically affect our conclusions.

Tuberculosis control

a. Patients

After tuberculin tests all positive inmates were subjected to clinical and roentgenological examination by fluoroscopy or X-ray at tuberculosis dispensaries or to micro X-ray photography. The tuberculosis suspects were then isolated from the others. All new arrivals were also tuberculin-tested and examined radiologically before they were placed in the wards.

Of the 226 tuberculin-positive patients 11 revealed clinically active tuberculosis; 3 of them, who died later, were bacillus carriers. Of these 11 patients two were treated at sanatoria and the others were isolated in the institutions.

Not one of the tuberculin-negative patients who before BCG vaccination were retested after an interval of 2 to 6 months had become tuberculin-positive during this time. The risk of TB infection from the positive patients, therefore, can be regarded as definitely eliminated.

b. Staff

The entire staff was examined roentgenologically at the beginning of the investigation by tuberculosis specialists thanks to the courtesy of our dispensaries. Not a single case of tuberculosis was found. The principle followed subsequently was that newcomers to the staff were examined roentgenologically on appointment unless they had been checked up shortly before, and that the staff as a whole, in addition, was examined annually by tuberculosis specialists. Sputum samples were taken in 6 cases, all with negative results. The staff employed during the term of the investigation totalled 605.

These arrangements, not always easy to assess, were considered so important and essential to the investigation that we consider we can claim that *our material of mentally defective children and young persons has really lived in a tuberculosis-free milieu.*

Technique

Technique of tuberculin tests

The first tests employed were the patch test for children from 0–7 years and Mantoux 0.1 mg for the older. In the event of a negative result Mantoux 1.0 mg was applied. At the beginning of the investigation the negatives were then BCG vaccinated. Since 1951, however, Mantoux 1.0 mg has been repeated on the negatives after 2–6 months, and only after this have they been BCG vaccinated.

The results of the vaccination were checked by the customary follow-up examination 3 months after vaccination. 6 months after the vaccination the tuberculin testing was repeated at half-yearly intervals. The examination was carried out to the following scheme: Mantoux 0.01 mg (1:10,000), Mantoux 0.1 mg (1:1,000) and Mantoux 1.0 mg (1:100). All these tests were made at one session on the skin of the back between the shoulder blades, with a minimum distance of 6 cm between each site of injection. We employed Old Tuberculin from the State Serum Institute in Copenhagen and the solutions were prepared by accurate pipetting the day the injections were given. Efforts were made to secure an exact injection technique with No. 20 needles; readings were taken 72 hours after injection, the reaction being computed by measuring two diameters at right angles to one another. All the Mantoux injections were made and their readings taken by one of us (M. D.), care being taken to follow the principles reported before (WASZ-HÖCKERT).

Any possible sign of Koch's phenomenon after vaccination has been studied. Similarly, the BCG scar was observed and its size noted.

Technique of BCG vaccinations

The BCG vaccine was received from the Bacteriological Laboratory at Sahlgrenska Sjukhuset, Gothenburg. This vaccine contains 0.5 mg of BCG organisms per ml. The vaccine was 5–7 days old, never older than 10 days. No bottle of vaccine was used on more than one occasion. The inoculation was made intracutaneously on the left thigh, with 0.1 ml vaccine; hence the injection contained 0.05 mg of BCG organisms.

Dates of BCG vaccination

Not all the 227 inmates were vaccinated at one time. The material increased annually. For this reason only 102 patients have been followed up for $3^1/_2$ years, the others for a shorter period.

TABLE 3

Annual total vaccinated, and the vaccine employed.

	1949	1950	1951	1952	Total
Number vaccinated	69	88	42	28	227
BCG vaccine No.	45, 51	2, 3, 4, 9	2, 4, 5, 12, 21, 36, 37, 38, 50	11, 13, 15, 17, 19, 21, 45, 49, 97	24 bottles

Results

Tuberculin-sensitivity after vaccination

Of the BCG vaccinated 8 were tuberculin-negative ad 1.0 mg Mantoux at the check-up, a percentage of 3.6. Two revealed Koch's phenomenon. These two plus the negative cases have been excluded from the material, which therefore totalled 217.

The strength of the tuberculin reactions after vaccination can be seen from the following table:

TABLE 4

Number of positive reactions in Mantoux groups, percentages.

	Mantoux 0.01 %	Mantoux 0.1 %	Mantoux 1.0 mg %
3 months	28.2	73.8	96.4
6 months	19.1	63.5	94.2

Disappearance of the sensitivity to tuberculin

The criterion adopted for negative sensitivity to tuberculin was a reaction which showed less than 10×10 mm infiltration and reddening 72 hours after 1.0 mg Mantoux (strictly according to WALLGREN), i.e. the same as in a previous investigation by one of us (WASZ-HÖCKERT). The results are given in Table 5, which indicates the number of persons who became negative during the period of the investigation, i.e. whose tuberculin-allergy had disappeared.

TABLE 5

Disappearance of sensitivity to tuberculin at various periods after BCG vaccination.

Time from vaccination, years	½	1	1½	2	2½	3½
Total examined	217	190	174	162	141	102
Total negative	4	14	17	26	40	30
Percentage of negative .	1.8	7.3	9.8	16.0	28.3	29.4

Although for both practical purposes and in theory the above criterion may be considered adequate—the sensitivity to tuberculin is so low that re-vaccination is necessary in any case, particularly if we bear in mind that the weaker reactions to Mantoux 1.0 mg may contain some element of pseudoreaction which the PPD is intended to eliminate—we selected another minimum limit, viz. 7 × 7 mm. The results are as follows:

TABLE 6

Number of negative reactions at various periods after BCG vaccination when negative reaction $\leq 7 \times 7$ mm.

Time from vaccination, years	½	1	1½	2	2½	3½
Total examined	217	190	174	162	141	102
Total negative	1	3	6	8	18	4
Percentage of negative .	0.4	1.6	3.4	5.0	12.7	4.0

The size of the tuberculin reaction at different dates after vaccination is given graphically in Fig. 1. The size of the infiltration is indicated in sq.mm and calculated as a mean value of the tuberculin reactions of all the examined to Mantoux solutions of varying concentration. The curve indicating

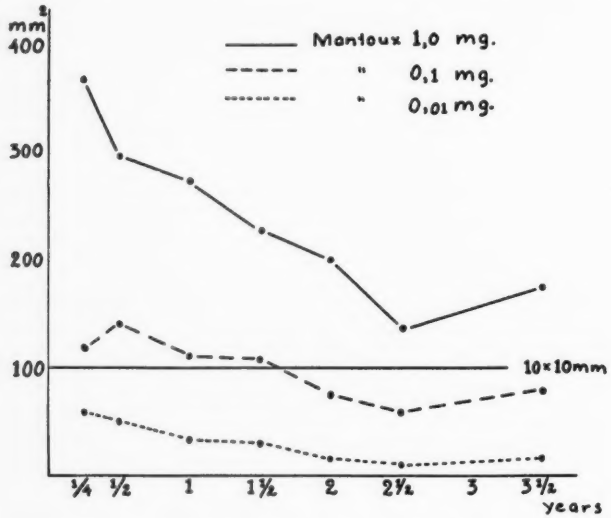

Fig. 1. Average size of tuberculin reactions at various periods after BCG vaccination.

TABLE 7

Occurrence and size of the BCG vaccination scar in the tuberculin-positive and tuberculin-negative group, $3\,^1/_2$ years after vaccination.

	Number examined	Occurrence of scar		Size of scar sq. mm
		Number	%	
Tuberculin-positive	72	72	100	37.8
Tuberculin-negative	30	29	96.7	31.0

10 × 10 mm reactions has been drawn in; it constitutes the borderline between tuberculin-positivity and -negativity to Mantoux 1.0 mg.

In the following tables (Tables 8 and 9) we have grouped the occurrence and size of vaccination scars by tuberculin-positive and tuberculin-negative cases in order to ascertain whether a possible relationship exists between allergy and local reaction.

TABLE 8

Size of the BCG vaccination scar in relation to the tuberculin sensitivity to 1.0 mg Mantoux.

Tuberculin reaction, sq. mm	Number examined	Size of scar, sq. mm
> 200	27	41.0
100—200	45	35.8
< 100	30	31.0

Discussion

The following table gives the results of the present and earlier investigations into the duration of allergy to tuberculin after BCG vaccination.

A study of the results in the above table reveals that the tuberculin-positivity induced by BCG vaccine has not been permanent except in the group examined by EDWARDS *et al.* The figures showing how soon the sensitivity to tuberculin disappears vary somewhat in the various materials. Probable contributory factors are (1) the risk of exposure of the material to tuberculosis, (2) the age distribution of the material, and individual factors, and (3) the criteria for negativity to tuberculin.

From Table 1 we find that there was a greater likelihood in the earlier investigations of the vaccinated being exposed to tuberculosis. This accounts for the fairly low percentages reported by DAHL, HERTZBERG and REFSUM, RYDÉN, and ANDERSON and BELFRAGE. RINVIK's investigation

TABLE 9

Number of persons no longer reacting to tuberculin, expressed as percentages of the total vaccinated, at certain dates after BCG vaccination.

Author	Date, indicated in years, after vaccination								Tub. test	Negativity limit
	½	1	2	3	4	5	6	7		
	%	%	%	%	%	%	%	%		
DAHL, HERTZBERG, REFSUM		0.6	2.4		3.2				Mantoux 1.0 mg	10 × 10 mm
RYDÉN			1.6	6.9					,,	,,
ANDERSON & BELFRAGE		3.2	4.7		2.1				,,	,,
RINVIK		66	90						,,	,,
ENELL							5	10	,,	9 × 9 mm
WASZ-HÖCKERT		7.5	9	9.3	11.3	14.9	17.5	9.7	,,	10×10mm
TÖRNELL					18.5				,,	,,
BLUHM	26.4	25	18.7	11	7.2	8.3			,,	,,
GEDDE-DAHL	16	41	40	25					Pirquet	
EDWARDS, PALMER, MAGNUS			0						10 TU	
WASZ-HÖCKERT & DONNER	1.8	7.3	16.0	29.4 %					Mantoux 1.0 mg	10 × 10 mm

has a special character in that his experimental subjects were definitely isolated from tuberculous infection and only comprised newborn babies.

The figures reported by ENELL, WASZ-HÖCKERT, TÖRNELL and BLUHM are in good agreement; all these investigations were carried out in Sweden (that of TÖRNELL at Borås, the others in Stockholm). If we study only that part of the material which according to the author has not been exposed to tuberculous infection, we obtain the following figures for the disappearance of Mantoux reactions:

TÖRNELL	18.5 per cent neg.
BLUHM	18.7 ,, ,, ,,
WASZ-HÖCKERT	17.4 ,, ,, ,,

Since the size of material and observation periods vary, the results are not comparable in detail.

It is difficult to compare GEDDE-DAHL's results with the others as the author employed the Pirquet method for the tuberculin test.

Contradicting all the other investigations is the one published by EDWARDS, PALMER and MAGNUS. In the course of a two-year observation period there was no reduction in the diameters of the reactions to tuberculin. Similar results were obtained in control investigations with vaccine from other countries, e.g. Sweden. The Danish school children were not isolated but the risk of tuberculous infection was considered extremely small by the authors.

Our own results from Finland show that tuberculin allergy following BCG vaccination disappears in a higher percentage of cases than has generally been assumed from earlier investigations. We have employed a BCG vaccine from Sweden, and our vaccination technique and principles in making the tuberculin tests concur with those employed in the Scandinavian investigations. We are of the opinion that virulent tuberculous infection has been excluded here and that the pure immunization conditions of the vaccination have been better revealed.

Between the two latest examinations, 2 $^1/_2$ and 3 $^1/_2$ years after the vaccination, there is an obvious increase in the tuberculin allergy. The reason for this increase has not been stated. Possibly facts such as a generally better state of health, the beginning of puberty etc. may influence and increase the ability to react. Such an influence will be more obvious as the allergy is already rather weak.

As pointed out by e.g. TÖRNELL, the occurrence of a BCG scar seems to indicate stronger and more prolonged tuberculin allergy. This could, however, not be significantly proved in our material, but as seen in Table 8 there is a tendency in this direction.

Summary

A preliminary report is given on investigations into the duration of sensitivity to tuberculin of 217 BCG vaccinated inmates of closed institutions. Both the inmates and the staff were under strict observation for tuberculosis. The results obtained are as follows:

1. The isolated milieu has been effective in excluding the risk of tuberculous infection. No increased allergy suggestive of super-infection was observed.

2. The patients examined were vaccinated with BCG vaccine from the Bacteriological Laboratory of Sahlgrenska Sjukhuset, Gothenburg. 0.1 ml containing 0.05 mg of BCG organisms was injected intracutaneously into the left thigh. 96.4 per cent of those vaccinated were positive at the check-up.

3. The tuberculin reaction has been taken as negative if smaller than 10 × 10 mm 72 hours after the injection of 1.0 mg Mantoux.

4. Two cases in which Koch's phenomenon was observed were excluded from the material.

5. After 1 year 7.3 per cent had become negative, after 2 years 16.0 per cent, after $2^1/_2$ years 28.3 per cent and after $3^1/_2$ years 29.4 per cent.

6. Mantoux test 0.01, 0.1 and 1.0 mg on the vaccinated showed that allergy to tuberculin, on the whole, declined with time, which supports the above result.

Persistance de la sensibilité à la tuberculine ches des personnes vaccinées par le B.C.G. et isolées dans des institutions.

Les auteurs donnent les premiers résultats de leur recherches sur la durée de la sensibilité à la tuberculine chez 217 pensionnaires d'institutions fermées et ayant été vaccinés par le B.C.G. Pensionnaires et personnel ont été soigneusement observés du point de vue tuberculose. Les résultats suivants ont été obtenus: Le milieu ainsi isolé n'a réellement présenté aucun risque d'infection tuberculeuse. On n'a pu remarquer aucune augmentation de l'allergie faisant penser à la possibilité d'une réinfection. Les sujets examinés ont été vaccinés par du B.C.G. provenant du Laboratoire bactériologique de l'hôpital Sahlgrenska à Gothenburg. Ils ont reçu par voie sous-cutanée dans la cuisse gauche 0,1 ml contenant 0,05 mg d'organismes B.C.G. 96,4 % des vaccinés ont été positifs lors du controle. La réaction à la tuberculine a été considérée comme négative lorsqu'elle était inférieure à 10 × 10 mm, 72 heures après l'injection de 1 mg de solution de Mantoux. Deux cas dans lesquels on avait observé un phénomène de Koch ont été exclus de la série. Après 1 an, 7,3 % étaient devenus négatifs, après 2 ans 16 %, après $2^1/_2$ ans 28,3 %, et après $3^1/_2$ ans 29.4 %. L'intradermoréaction de Mantoux réalisée avec des solutions de 0,01, 0,1 et 1 mg chez les vaccinés a permis de constater que l'allergie diminuait dans les limites de temps indiquées par les résultats ci-dessus.

Die Dauer der Tuberkulinempfindlichkeit bei BCG-vaccinierten Personen in geschlossenen Institutionen.

Es wird ein vorläufiger Bericht über die Tuberkulinempfindlichkeit von 217 BCG-geimpften Personen in geschlossenen Institutionen gegeben. Sowohl die Insassen als auch das übrige Personal standen unter strenger Tuberkulosekontrolle. Die erhaltenen Resultate waren folgende: Das isolierte Milieu konnte wirksam das Risiko einer tuberkulösen Infektion ausschließen. Es wurde keine auf Superinfektion verdächtige gesteigerte Allergie beobachtet. Die untersuchten Patienten wurden mit BCG-Impfstoff aus dem bakteriologischen Laboratorium des Sahlgrenschen Krankenhauses in Göteborg vacciniert; 0,1 cm^3, der 0,5 mg BCG-Organismen enthält, wurde intracutan in die linke Hüftgegend injiziert. 96,4 % der Geimpften waren bei der Nachprüfung positiv. Die Tuberkulinreaktion mit 1,0 mg Mantoux galt als negativ, wenn sie nach 72 Stunden kleiner als 10 × 10 mm war. Zwei Fälle mit positiven Koch' schen Phänomen wurden aus dem Material ausgeschlossen. Nach 1 Jahr waren 7,3 % negativ, nach 2 Jahren 16 %, nach $2^1/_2$ Jahren 28,3 % und nach $3^1/_2$ Jahren 29,4 %. Der bei den Geimpften durchgeführte Mantoux-Test mit 0,01, 0,1 und 1,0 mg zeigte, daß die Tuberkulinallergie mit der Zeit abnimmt, was die obigen Resultate stützt.

Persistencia de la sensibilidad a la tuberculina en personas vacunadas con el B.C.G. y aisladas en instituciones.

Una relación preliminar de las investigaciones respecto a la duración de la sensibilidad a la tuberculina de 217 personas B.C.G. vacunadas en instituciones cerradas. Tanto esas personas como el staff eran cuidadosamente observados por tuberculosis

Los resultados: El aislamiento ha sido efectivo en cuanto a suprimir el riesgo de la infección tuberculosa. No se observé aumento de la alergía sugestivo de una superinfección. Los enfermos examinados fueron vacunados con B.C.G. del laboratorio bacteriológico del hospital Sahlgrenska de Gotemburgo. 0,1 ml conteniendo 0,05 mg de organismos B.C.G. fueron inyectados intracutanamente en la pierna izquierda. El 96,4 % de los vacunados fueron positivos al control. La reacción tuberculínica fué considerada negativa si era menor de 10 por 10 mm 72 horas después de la inyección de 1,0 mg de Mantoux. Dos casos en los que se observó el fenómeno de Koch fueron excluídos del material. Después de un año el 7,3 % de los casos se volvió negativo, luego de dos años el 16 %, después de 2 años y medio el 28,3 % y después de 3 años y medio el 29.4 %. El test de Mantoux al 0,01, 0,1 y 1,0 mg en los vacunádos, mostró que la alergia a la tuberculina declina con el tiempo.

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The Dosage of Chloromycetin Palmitate in Children

by HANS-OLOF MOSSBERG

Crystalline chloromycetin (chloramphenicol) is an intensely unpalatable powder, enclosed in capsules. In children unable to swallow the capsules the administration of this drug is a difficult problem. The repulsive taste produces negative reactions, nausea and not infrequently vomiting, which may entirely jeopardize the utilization of the drug in these children.

Parke-Davis' new chloromycetin preparation, chloromycetin palmitate, consists of an ester with a 57.6 % chloromycetin base. The palmitate is palatable. In the bowel it is split by the lipase of the pancreatic and intestinal juices and is then absorbed by the blood in its free form of chloramphenicol. Palmitate as such does not possess antibiotic action but gains this after being split in the bowel (ROSS, BURKE and RICE).

Hydrolysis of the chloromycetin palmitate in the bowel requires the presence of adequate amounts of lipase. Investigations by ROSS, BURKE and RICE, in a case of pancreatic fibrosis with a markedly changed secretion of pancreatic enzymes, establish that the bowel content of lipase, notwithstanding this, is sufficient to split the chloromycetin palmitate. These authors have also established that children with diarrheal conditions are able to split and absorb the chloromycetin palmitate satisfactorily.

Thanks to the courtesy of Parke-Davis it has been made possible to test chloromycetin palmitate at Barnsjukhuset Samariten.

Material and Methods

Thirty-one children, in ages ranging from 1 month to 5 years, were studied, consideration being paid to their reactions to the administration of crystalline chloromycetin and of chloromycetin palmitate. The children were given the two media three days in succession and their reactions were registered. The crystalline chloromycetin was given first in one-half the cases, while the palmitate was given first to the remainder. The taste of the crystalline chloromycetin was, as far as possible, rendered less repulsive by adding lemon juice and sugar. As appears from Fig. 1 the reactions are divided into positive and negative ones.

The rate of adsorption to the blood and the blood concentrations were tested with consideration to both drugs. Three children in an age ranging from 3 to 9 years were

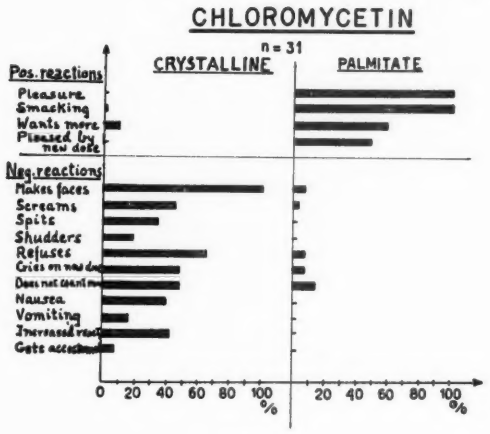

Fig. 1.

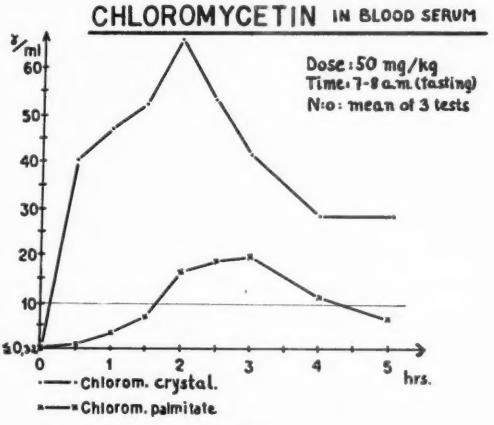

Fig. 2.

given fasting a single dose of 50 mg/kg body weight in the morning, one day, and the same dose in the afternoon 2 hours after dinner, another day (Figs. 2 and 3). The blood concentration was determined prior to, and every 30 minutes up to 3 hours after, the administration of the chloromycetin, and subsequently once an hour up to 5 hours after the beginning of the test. The chloromycetin concentration in the blood was determined microbiologically in micrograms (γ) active chloromycetin per ml blood serum.[1]

[1] The chloromycetin determinations were carried out by Dr. Brita Ericson at *Sjukhusdirektionens Bakteriologiska Laboratorium*, Stockholm.

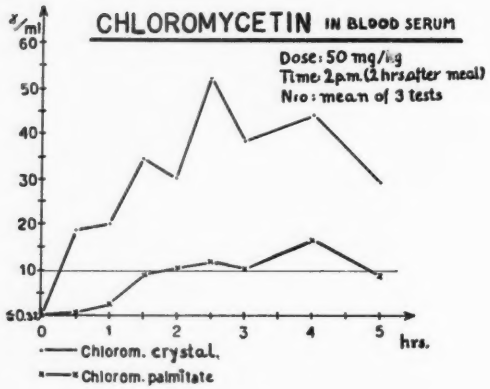

Fig. 3.

TABLE 1

Chloromycetin, mean max. value (γ/ml) after single dose.

Time	Crystalline		Palmitate
	50 mg/kg	15 mg/kg	50 mg/kg
7—8 (fasting)	66.7	17.5	19.4
14 (2 h. after meal)	52.1	12.5	16.8
	$n = 3$	$n = 2$	$n = 3$

At similar times of day two children were given crystalline chloromycetin in a single dose of 15 mg/kg body weight. The maximum values obtained in these tests are shown in Table 1.

In order to ascertain the most appropriate dose of chloromycetin palmitate in continuous treatment 5 children were given 50 mg/kg body weight 4 times in 24 hours (8 a.m., 2 p.m., 8 p.m., and 2 a.m.,) and 5 other children were given 25 mg/kg body weight at the same time intervals. Both groups were constituted by children 4–7 years of age. Blood samples were taken within 30 minutes prior to each repeated chloromycetin dose, excepting at night (Table 2). In two children the trial was continued for another 48 hours in order to follow the variation in the blood concentration values during prolonged treatment.

Results

The difference in the children's reaction to the administration of crystalline chloromycetin and of chloromycetin palmitate appears from Fig. 3. The superiority of the palmitate is unequivocal. In the youngest infants the negative reaction towards crystalline chloromycetin was rather weak but

TABLE 2

Chloromycetin, mean blood concentration value (γ/ml) immediately prior to new dose in continuous treatment (8 a.m., 2 p.m., 8 p.m., 2 a.m.).

Time of test	Palmitate	
	50 mg/kg	25 mg/kg
13.30—14.	2.5	≦ 1.1
19.30—20.	4.3	≦ 3.4
7.30—8	13.2	5.8
	$n = 5$	$n = 5$

increased in intensity with the age of the children. In the oldest children (3–5 years) the intensity of the negative reactions was somewhat mitigated by their power of self-control.

The absorption rate for crystalline chloromycetin and chloromycetin palmitate, and the maximum blood concentration values for the two drugs are presented in Figs. 2 and 3. The rate of absorption is essentially more rapid for crystalline chloromycetin than for chloromycetin palmitate. The maximum values of the blood concentration on the fasting administration of crystalline chloromycetin (Fig. 2) are reached about 1 hour earlier and are 3 times higher than on the administration of chloromycetin palmitate. The maximum value for crystalline chloromycetin is obtained 2 hours after the beginning of the test. When the tests are performed after a meal (Fig. 3) the adsorption of both drugs is delayed and the maximum blood concentration values are slightly lower than in the fasting tests. The maximum value for crystalline chloromycetin is thereby obtained after 2 $^1/_2$ to 3 hours. As late as 5 hours after beginning the test the blood concentration was about 30 γ/ml for crystalline chloromycetin with the aforementioned dosage, while the comparable value for chloromycetin palmitate was 5–10 γ/ml.

In Table 1 are presented the maximum blood concentration values in the tests described above. When 15 mg/kg (approx. 1/3 of the original dose of crystalline chloromycetin) is administered the maximum values obtained are in good agreement with the maximum values of chloromycetin palmitate in a dosage of 50 mg/kg. The dosage of chloromycetin palmitate should thus be 3 times as great as the dosage of crystalline chloromycetin in order to afford maximum blood concentrations of the same order of magnitude.

In Table 2 are shown the results of continuous administration of chloromycetin palmitate. The values show that there is a successive rise of the blood concentration during the 24-hour test. With the greater dosage of chloro-

mycetin palmitate (50 mg/kg × 4) the mean blood concentration 5 $^1/_2$ to 6 hours after the initial dose was low, 2.5 γ/ml, and with the smaller dose (25 mg/kg × 4) ≦ 1.1 γ/ml. In the morning after 24 hours of administration the comparable values were 13.2 and 5.8 γ/ml, respectively. In a few cases with prolonged administration the morning values after 48 and 96 hours were about the same. During daytime, however, there is a lowering of the blood concentration to a minimum immediately before administration of the next dose of chloromycetin palmitate. The minimum values found are about one-fourth the fasting value in the morning.

Discussion

In Fig. 1 the superiority of chloromycetin palmitate was established as compared with crystalline chloromycetin with regard to the children's positive and negative reactions on peroral administration. In children unable to swallow capsules, chloromycetin palmitate is thus a valuable drug in chloromycetin treatment.

Intestinal disturbances giving rise to imperfect hydrolysis of the chloromycetin palmitate and deficient absorption of the chloromycetin are rare. The absorption curves in the present study (Figs. 2 and 3) show that the chloromycetin palmitate should be given with a single dose of approx. 15 mg/kg body weight crystalline chloromycetin. Hereby the blood concentration of chloromycetin reaches a therapeutically active blood rate more rapidly. The importance of this also appears in the experiments with the object of determining an appropriate continuous dosage of chloromycetin palmitate (Table 2).

On the administration of crystalline chloromycetin in capsules it takes 15 minutes at the most to dissolve the capsule in the stomach. Thus, the capsule retards the absorption of chloromycetin but insignificantly. The absorption curves for crystalline chloromycetin with and without capsule are practically identical.

Most microorganisms sensitive to chloromycetin require a blood concentration of approx. 5 γ/ml. It is accordingly a desideratum to keep the blood concentration of chloromycetin above this level, thus for example at 10 γ/ml. In determination of an appropriate continuous dose of chloromycetin palmitate (Table 2) the blood concentration in the morning after 24 hours' treatment with 50 mg/kg × 4 = 200 mg/kg showed approx. mean values of 13 γ/ml, and with 25 mg/kg × 4 = 100 mg/kg the mean value was approx. 6 γ/ml with a lowering of the values during daytime to about one-fourth of the value in the morning. The appropriate dose of chloromycetin palmitate for the maintenance of a minimum blood concentration of approx. 5 γ/ml

is thus 200 mg/kg per 24-hour period, administered in 4 doses. This dosage is higher than that recommended by Ross *et al.* and by Yow *et al.* These authors, however, have not taken notice of the lowest values of blood concentration during 24 hours.

Summary

Parke-Davis' palatable chloromycetin palmitate was tested. It is easily taken by young children, in contrast to the extremely repulsive crystalline chloromycetin (chloramphenicol). The absorption rate for chloromycetin palmitate is slower than that for crystalline chloromycetin and the maximum blood concentration, which for crystalline chloromycetin is obtained about 2 hours after the fasting administration, is not reached until 1 hour later for chloromycetin palmitate. A further retardation of absorption takes place if the drug is not administered fasting. The maximum blood concentration of crystalline chloromycetin is three times higher than the maximum values for the palmitate of the same dosage. Control experiments show that the chloromycetin palmitate dosage should be three times greater than that of crystalline chloromycetin in order to obtain the same maximum blood concentrations. For the maintenance of a *lowest* blood concentration of chloromycetin of about 5 γ/ml, a chloromycetin palmitate dose of 200 mg/kg per 24 hours, administered in 4 doses, is required. In order to obtain a more rapid initial rise of the blood concentration the first dose of chloromycetin palmitate should be given with a single dose of crystalline chloromycetin (approx. 15 mg/kg).

La dose de palmitate de chloromycétine aux enfants.

Le palmitate de chloromycétine Parke-Davis, plus agréable au goût, est beaucoup plus facilement accepté par les enfants que la désagréable chloromycétine cristallisée (Chloramphenicol). Le taux d'absorption du palmitate de chloromycétine est moins élevé que celui de la chloromycétine cristallisée. La concentration sanguine maximum atteinte 2 heures après l'absorption, à jeun, de la chloromycétine cristallisée, ne l'est qu'une heure plus tard, avec le palmitate. Le retard de l'absorption est encôre plus marqué, si le médicament n'est pas absorbé à jeun. Pour une même dose, la concentration sanguine maximum est trois fois plus élevée avec la chloromycétine cristallisée qu'avec le palmitate. Des expériences de contrôle ont montré qu'il fallait un dosage triple de palmitate pour atteindre la même concentration sanguine qu'avec la chloromycétine cristallisée. Une dose de palmitate de chloromycétine de 200 mg par kg et par 24 heures administrée en 4 fois, est nécessaire pour maintenir une concentration sanguine très basse, de l'ordre de 5 γ par ml. Dans le but d'obtenir une élévation plus rapide de la concentration sanguine, la première dose de palmitate pourra être administrée simultanément avec une dose de chloromycétine cristallisée (environ 15 mg par kg).

Die Dosierung des Chloromycetin palmitate für Kinder.

Parke-Davis' wohlschmeckendes "Chloromycetin palmitate" wurde geprüft. Im Gegensatz zu dem übelschmeckenden kristallisierten Chloromycetin (Chloramphenicol), kann es ohne Schwierigkeit kleineren Kindern gegeben werden. Chloromycetin palmitate wird langsamer resorbiert als kristallisiertes Chloromycetin. Die maximale Blutkonzentration ist beim kristaillsierten Chloromycetin etwa 2 Stunden nach der

Nüchternapplikation erreicht, während es beim Chloromycetin palmitate erst eine Stunde später zur Höchstkonzentration kommt. Die Resorption kann weiterhin verzögert werden, wenn das Mittel nicht in nüchternen Zustand gegeben wird. Bei derselben Dosierung ist die maximale Blutkonzentration des kristallisierten Chloromycetins 3mal so hoch wie die des Chloromycetin palmitate. Kontrollversuche haben gezeigt, daß man zum Erreichen der gleichen maximalen Blutkonzentration eine Chloromycetin palmitate Dosis geben muß, die 3mal so groß wie die des kristallisierten Chloromycetins ist. Um die niedrigste Chloromycetinkonzentration im Blut aufrechtzuerhalten (etwa 5 γ/ccm), muß man 200 mg/kg/24 Std. in 4 Dosen aufgeteilt geben. Wünscht man einen schnelleren Anstieg der Blutkonzentration, soll man die erste Dosis Chloromycetin palmitate mit einer Einzeldosis von kristallisierten Chloromycetin kombinieren (etwa 15 mg/kg).

La dosis de palmitato de chloromicetina por los niños.

El palmitato de cloromicetina es mucho más fácilmente aceptado por los niños que la desagradable cloromicetina cristalizada. El índice de absorción del palmitato de cloromicetina es menos elevado que el de la cloromicetina cristalizada. Mientras que la concentración sanguínea máxima de la cloromicetina cristalizada es alcanzada dos horas después de la absorción en ayunas, para el palmitato ocurre 1 hora después. El retardo de la absorción es todavía más marcado si el medicamento no se absorbe en ayunas. Para una misma dosis la concentración sanguínea máxima es tres veces mas elevada con la cloromicetina cristalizada que con el palmitato. Las experiencias de control han demostrado que es necesaria una dosis triple de palmitato para alcanzar la misma concentración sanguínea que con la cloromicetina cristalizada. Se necesita una dosis de palmitato de 200 mg por kilo cada 24 horas administrada en 4 veces, para mantener una concentración sanguínea baja del orden de 5 por ml. Para obtener una elevación más rápida de la concentración sanguínea la primera dosis de palmitato puede ser administrada con una dosis de cloromicetina cristalizada (15 mg por kilo).

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Iron Metabolism in Infants

II. Absorption of Dietary Iron

by Y. M. FEUILLEN

Historical

As early as 1889, BUNGE believed that only organic compounds of iron were absorbable by the intestinal wall; on the other hand SCHIROKAUER, STARKENSTEIN, and LINTZEL thought that only the inorganic salts were absorbable. Nevertheless, the more recent works of HAHN *et al.*, OLDHAM, BLACK and POWEL have shown that organic iron can be assimilated.

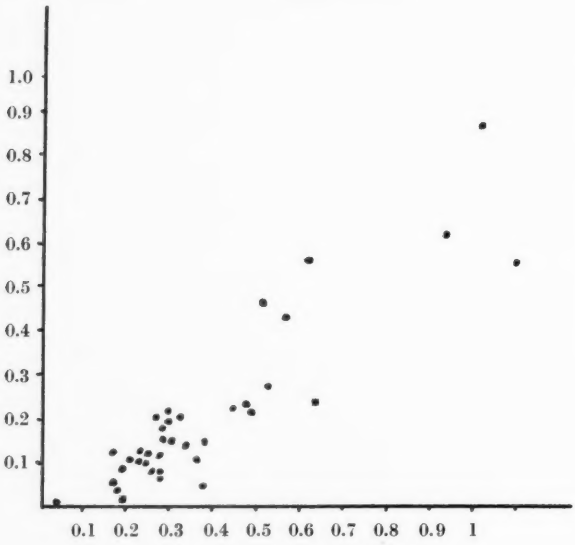
The experimental proceedings employed for studying absorption are as follows:

1. — The study of absorption of an iron preparation, rendered radioactive, by a portion of isolated intestine (8).

2. — The increase in quantity of blood haemoglobin after iron therapy (1, 20). This method does not allow the quantitative measurement of absorption. It would be preferable to separate the study of absorption from that of utilization of iron.

3. — The study of absorption by estimation of the plasma iron before and after administration of different forms of medicinal iron. By this method HEILMEYER, MOORE *et al.*, VAN GOIDSENHOVEN *et al.*, COSYNS etc. concluded that iron can only be assimilated in its mineral form, bivalent and ionized.

According to PLUMIER, when performing absorption tests in a case of hemochromatosis, an iron balance carried out at the same time as an absorption test by estimation of plasma iron, shows a definite retention whereas the absorption test is negative. Similarly, LAMBRECHTS administered iron to adult subjects, on the one hand through the stomach, and, on the other, directly into the duodenum (Einhorn tube). In the former case, the serum iron curve showed a greater and more rapid increase than in the case of administration through the duodenum. Moreover balances carried out in both cases showed comparable results.



Abscissae: Intake (mg/kg/day)
Ordinates: Retention (mg/kg/day)

Fig. 1.

If increase of plasma iron is a proof of absorption, the fact that the plasma iron is not increased does not establish non-absorption.

4. — The balance method has been used in the present experiments. This technique can be criticized on the ground that the fecal iron represents the unabsorbed iron, plus the iron excreted by the bile, the excretion by the intestine being negligible. In spite of this fact, we have been able to use this method, considering that the percentage absorptions, as they appear in our balances, are in fact minimum figures; the relatively high figures that we obtain are therefore especially significant.

5. — Another satisfactory method is the study of absorption by means of radioactive iron. The method of Hahn, also used by other authors (Darby *et al.*, Moore *et al.* and Oettinger *et al.*), consists in finding what percentage of ingested radioactive iron is recovered in the newly formed blood cells. The same objection already quoted above with respect to the increase in the quantity of hemoglobin after iron therapy, is valid for this procedure.

6. — Finally, Mac Cance and Widdowson, studying the case in man, have calculated the increase of iron in the whole body during the lactation period using data obtained from the iron content of different tissues. This proceeding is an indirect one and yields only hypothetical results.

TABLE 1

No.	Intake, mg per kg per day	Retention, mg per kg per day	Retention, %
1	0.55	0.42	76
2	1.08	0.55	51
3	0.32	0.20	63
4	0.19	0.08	45
5	0.26	0.20	78
6	0.51	0.46	91
7	0.16	0.12	73
8	0.61	0.56	92
9	1.00	0.86	86
10	0.92	0.61	66
11	0.48	0.23	47
12	0.27	0.06	25
13	0.63	0.23	36
14	0.28	0.18	64
15	0.33	0.14	43
16	0.29	0.21	72
17	0.37	0.04	12
18	0.03	0	0
19	0.30	0.15	50
20	0.29	0.19	66
21	0.17	0.05	32
22	0.24	0.10	41
23	0.44	0.22	50
24	0.19	0	0
25	0.26	0.08	31
26	0.36	0.10	27
27	0.48	0.21	44
28	0.23	0.12	53
29	0.27	0.07	25
30	0.25	0.11	45
31	0.25	0.07	28
32	0.52	0.27	53
33	0.29	0.15	53
34	0.37	0.14	37
35	0.22	0.10	47
36	0.28	0.12	43
37	0.22	0.10	48
Means	0.37	0.19	53

The most suitable method seems to be the balance method in combination th use of labelled iron. This technique will be used later on to complete the ta of our first series of balances.

Having shown (6) that infants artificially fed ingest up to 3 or 4 times more iron than breast fed infants, we propose now to investigate in what measure this supplementary quantity of iron is absorbed by the intestine.

Methods

In this first series, we investigated 19 full-term infants from the age of 15 days to 9 months. At the time, they showed no fever, infection, intestinal disturbance, nor anaemia; nevertheless, a certain number of them were more or less hypotrophic. For several days before the beginning of the experiment, these infants received a known volume of the following diets:

(1) mixture of milk, water, saccharose, dextrimaltose or starch.
(2) buttermilk and acid milk made in our own diet kitchen.
(3) commercial acid milk (Nestlé).
(4) commercial buttermilk (Nestlé).
(5) mixture (1) plus vegetable soup (fresh or tinned).

Each balance experiment lasted 3 days. The amount of iron in the intake was never calculated from tables or on the basis of mean values from our previous estimations. Actually, the possibilities of iron contamination during the preparation of the diet are such that only estimations repeated every day, on a sample taken directly from the bottle, avoid this important cause of error.

As it has been established that renal excretion of iron is practically negligible in normal conditions, urine was not collected. In collecting the faeces, great care must be taken to avoid the use of talcum powder during the baby's toilet, as this introduces marked errors in the balance. The child is diapered on a porcelain pot fitted into a plastic-covered mattress. This procedure allows the collection, without discomfort to the subject, of the total amount of the feces without contact with the diaper or the buttocks of the infant.

The total feces are dried at 100° to constant weight, then powdered and well mixed before sampling. The iron estimations in the diet and the faeces are carried out by the O-phenantrolin method after wet ashing with sulfuric and perchloric acid; the readings are taken on the Lumetron electro-colorimeter after 24 hrs. standing. The results are expressed in mg per kg body weight per day, which enables us to compare the absolute values of intake and retention when the weights of the infants differ considerably.

Results

The results of our 37 balances are set out in Table 1 and Fig. 1.

Discussion

As indicated in the Table, there are extensive variations in absolute intake and retention values as expressed in mg per kg per day. This is explained as much by the variety of diets administered and the iron value contained therein, as by the different body weights of the subjects. If these results are plotted on a graph one fact is obvious: there is a relation between the amou

of ingested iron and absorbed or retained iron. This shows fairly variable percentage absorption, which nevertheless fluctuates about 50 %, whatever the amount of iron. According to our results, it appears that the intestine absorbs approximately half of the alimentary iron, at least within the limits of the quantities of iron contained in the usual diets.

It is emphasized that in this series of 37 experiments on 19 infants, we have had only 2 zero balances and none that were negative. We cannot confirm either, the results of STEARNS and STINGER who obtained negative balances in infants between the ages of 7 weeks and 1 year, receiving acid milk with carbohydrates and apple juice, or those of MAURER and GREENGARD who found 9 times out of 12 a negative balance on artificial diets. Our data are near those obtained by JOSEPHS who found in a large number of infants, fed a mixture of milk and cereals, an absorption of about 60 %. On the other hand we could not observe the unfavourable influence of early age on iron absorption, to which this author makes reference.

OLDHAM *et al.* who, in 28 balance experiments, studied the influence of the nature of inorganic or organic iron compounds on their retention, obtained an absorption less than 19 %. Unfortunately these results have been obtained on one infant only between the ages of 6 and 14 months.

Our data show that the iron contained in an artificial diet is properly assimilated by the infant, at least up to about 50 %; this amount corresponds to an average daily retention of 0.19 mg per kg body weight in the 19 subjects we studied. What amount of iron would these same infants have retained, had they been breast fed? Their body weight, on an average, was 5 kg and their diet about 720 g per day. If we consider that human milk contains about 50 γ % Fe (FEUILLEN and PLUMIER), we can calculate an average intake of $\frac{50 \times 7.2}{5} = 72 = 0.07$ mg Fe/kg/day. What percentage would be absorbed by the intestine? Unfortunately, we had very few opportunities of studying infants fed with breast milk: 2 balances were performed; the results are expressed in mg/kg/day.

Balance No. 53:	Intake:	0.08	Retention:	0.04	Retention, %:	48 %
Balance No. 57:	„	0.12	„	0.09	„	„	78 %

When one considers the important individual variations in our series of balances, it is obvious that we cannot draw conclusions from the results of 2 experiments: they have only an indicative value. WALLGREN, studying iron absorption during the first year of breast fed infants, obtained negative balances fairly frequently during the first five months. These, and our two experiments, indicate that the absorption of iron contained in human milk is not complete. Nevertheless, even supposing that our breast fed subjects

were able to retain 100 % of the iron taken in, their retention would only have been about 0.07 mg/kg/day, which is less than half of the iron retained (0.19 mg) from an artificial diet.

Previously, we have shown (6) that infants artificially fed ingest 3 to 4 times more iron than breast fed infants; now we show that this larger amount of iron is absorbed up to about 50 %, which represents an absolute retention even higher than that of an ideal 100 % retention of the iron of breast milk. The fact that anaemia is more frequent among artificially fed infants than among breast fed, is due neither to a lack of iron in the diet, nor to a deficient absorption.

Summary and Conclusions

Thirty-seven alimentary iron balances on 19 infants between the ages of 15 days and 9 months show that these subjects ingest an average of 0.37 mg of iron per day per kg, of which they retain an average of 0.19 mg, i.e. 53 %. Within the limits of the amounts used in these experiments, the retention was shown to be approximately proportional to the intake. Within the age limits, we have observed only 2 zero balances and no negative one.

Le métabolisme du fer chez le nourrisson. II. Absorption du fer de l'alimentation.

37 bilans alimentaires chez 19 nourrissons âgés de 15 jours à 9 mois montrent que ces sujets ingèrent en moyenne 0,37 mg de fer par jour et par kg de poids corporel, dont ils absorbent en moyenne 0,19 mg, soit 53 %. Dans les limites des doses administrées dans ces expériences, la rétention s'est montrée approximativement proportionnelle à l'ingestion. Dans ces limites d'âge, nous n'avons observé que deux bilans nuls et aucun bilan négatif.

Eisenstoffwechsel bei Säuglingen. II. Resorption von Nahrungs-Eisen.

Bei 19 Säuglingen im Alter von 15 Tagen bis zu 9 Monaten wurden 37 Eisenbilanzen durchgeführt. Die Zufuhr betrug durchschnittlich 0,35 mg Eisen/Tag/kg, wovon im Durchschnitt 0,19 mg (= 53 %) resorbiert wurden. In den Grenzen der in diesen Experimenten zugeführten Eisenmengen war die absorbierte Menge ungefähr der zugeführten Menge proportional. In diesen Altersgrenzen beobachteten wir nur 2 Null-Bilanzen und keine negative Bilanz.

El metabolismo del hierro en el lactante. II. Absorción del hierro de la alimentación.

37 balances alimenticios en 19 lactantes de 15 días de edad a 9 meses muestran que esos sujetos ingieren en término medio 0,37 mg de hierro por día y por kilo de peso corporal, de los cuales absorben por término medio 0,19 mg o sea 53 %. En los límites de las dosis administradas en estas experiencias, la retención es proporcional a la ingestión. En estos límites de edad, no hemos observado mas que 2 balances nulos y ninguno negative.

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(Prof. A. LAMBRECHTS)

Iron Metabolism in Infants

III. The Influence of Vitamin C on the Absorption of Iron

by Y. M. FEUILLEN and A. LAMBRECHTS

The influence of the state of iron—whether ferrous or ferric—on the rate of absorption by the intestine has been studied by many authors, chiefly because of the therapeutic results.

In close relation to this problem, the influence of vitamin C has been studied in several conditions. On the basis of experimental results obtained by various techniques (serum-Fe curve, use of radioactive iron, influence on infectious anaemia) different authors have concluded that Vitamin C (or the reduction to ferrous state) has a favourable influence on the absorption of iron.

We have taken up the study of this question, using the balance method, particularly in the case of infants.

Method

Fourteen determinations were made on 7 healthy infants from 4 to $7^1/_2$ months, divided into 2 groups: in five cases, a three-day balance was carried out firstly on the normal diet, followed immediately by another three-days period on the same diet, plus 100 mg of vitamin C per day; in two cases, 2 and 4 mg of iron (ferric citrate) per day were added to the diets before and during the first balance; during the second period, the same amounts of ferric citrate were given, plus 100 mg of vitamin C per day.

Sampling, ashing, and estimation of O-phenantrolin-iron complex were performed as previously mentioned (4).

Results

See Table 1 and Figure 1.

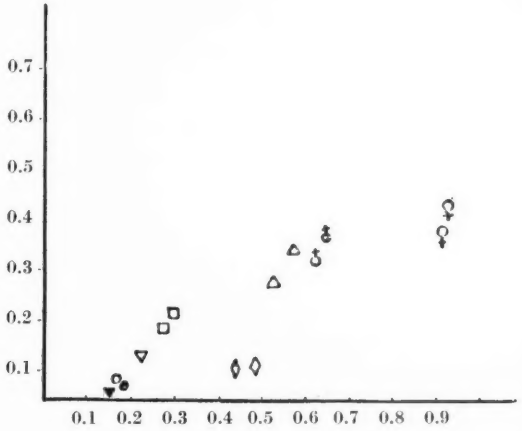
Discussion

It is interesting to analyse papers on this subject in relation to the methods used by different authors.

1. Quite a number have studied absorption by measuring the serum iron curve after administration of different forms of iron (HEILMEYER, MOOR

TABLE 1

Without Vitamin C					With Vitamin C				
No.	Intake mg		Retention mg	Retention %	No.	Intake mg		Retention mg	Retention %
	Diet	Suppl.				Diet	Suppl.		
1.	0.17	—	0.05	32	1a.	0.18	—	0.04	25
2.	0.52	—	0.27	53	2a.	0.57	—	0.34	59
3.	0.22	—	0.10	48	3a.	0.15	—	0.02	17
4.	0.27	—	0.17	63	4a.	0.29	—	0.20	68
5.	0.49	—	0.08	16	5a.	0.44	—	0.08	18
6.	0.22	0.40	0.32	52	6a.	0.24	0.40	0.37	57
7.	0.18	0.74	0.44	48	7a.	0.17	0.74	0.38	42
Mean	0.46		0.20	45		0.45		0.20	45



Abscissates: Intake (mg/kg/day)
Ordinates : Retention (mg/kg/day)

The white dots represent the results of balances without Vitamin C.
The black dots represent the results of balances with Vitamin C.

al., VAN GOIDSENHOVEN *et al.* and COSYNS). They established that the administration of ferrous iron produces a marked increase of serum iron, a phenomenon which is not reproduced after ingestion of ferric iron. These authors concluded that iron was absorbed by the intestine only in its bivalent form. We have already emphasized (4) how these conclusions should be interpreted: if such an increase in the plasma iron curve is a proof of absorp-

tion, the absence of increase does not exclude the possibility that absorption has occurred: although complete, absorption could occur more slowly or at different levels, thus giving an iron curve more spread out with time.

The same objection can be made to similar observations by BUGUEY, SCHRÖDER and BRAUN who observed an increase of serum iron after the administration of vitamin C. ALBERS eliminates possible modifications in the absorption of iron under the influence of vitamin C by injecting this directly: he observed in normal subjects an increase in serum iron. ALBERS concluded that vitamin C plays a role in the utilization of iron, a conception which in our opinion comes closer to the truth.

2. HAHN *et al.* and MOORE *et al.*, using bivalent and trivalent salts of radioactive iron, investigated the appearance of this element in the red blood cells of normal and anaemic dogs, and men. They found a better utilization of $Fe^{\cdot\cdot}$ than $Fe^{\cdot\cdot\cdot}$ for the regeneration of haemoglobin. MOORE *et al.* observed this phenomenon in man but not in the dog.

Here again, it seems to us that one should avoid confusion between utilization for haemoglobin and intestinal absorption.

3. Even more indirect, and less significant, is the study of the effects of vitamin C on infectious anaemia (BRAUN). By the same technique, SCHULZE and MORGAN (in 36 infants, divided into two groups, receiving respectively iron and copper, and iron, copper and vitamin C) observed the same increase in the rate of haemoglobin in both groups.

4. The balance method appears to be the only valid one. By this method, JOSEPHS ascertained that vitamin C influences absorption very little. Our results are in complete agreement with those of JOSEPHS: in infants we observed no influence of 100 mg vitamin C on the absorption of dietary iron and of small supplementary doses, within the physiological range. We did not investigate the possible effect of ascorbic acid on high "therapeutic" doses of iron.

Summary

Fourteen iron balances in 7 infants from 4 to $7\frac{1}{2}$ months show no influence of vitamin C on the intestinal absorption of dietary iron and of small doses of ferric citrate.

Métabolisme du fer chez le nourrisson. III. Influence de la vitamine C sur l'absorption du fer.

14 bilans métaboliques du fer, exécutés chez 7 nourrissons de 4 à 7,5 mois, montrent que la vitamine C n'exerce aucune influence sur l'absorption du fer alimentaire, ou de petites doses de citrate ferrique.

Eisenstoffwechsel bei Säuglingen. III. Einfluß des Vitamin C auf die Absorption des Eisens.

In 14 Eisenbilanzen, bei 7 Säuglingen im Alter von 4 bis 7,5 Monaten, hat das Vitamin C bei der Absorption des alimentären Eisens oder kleiner Eisencitratmengen keine Rolle gespielt.

Metabolismo del hierro en el lactante. III. Influencia de la Vit. C en la absorción del hierro.

14 balances del metabolismo del hierro en niños de 4 a 7 meses y medio muestran que la vitamina C no ejerce influencia en la absorción del hierro o de pequeñas dosis de citrato férrico.

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CASE REPORT

Congenital Valvular Formation of the Posterior Urethra

by K. H. TORP

From the University Hospital (Rikshospitalet), Oslo, Norway, Pediatric Clinic (Chief: Professor L. Salomonsen, M.D.) and Institute of Pathological Anatomy (Chief: Professor O. Torgersen, M.D.).

Congenital valvular formation of the posterior urethra in boys, with impeded urination and dilatation of the urinary tract, is a morbid picture familiar to pediatricians. When nevertheless a report is given on this lesion, it is to underline some factors and to recall that this anomaly is not rare.

The following four cases deal with male infants who were referred for post-mortem examination to the University Hospital (Rikshospitalet), Institute of Pathological Anatomy, in the course of six months.

Case Reports

Case 1. P.M. No. 98/52. Newborn male infant, about four weeks premature. Poor foetal heart sounds—delivered by forceps. The infant was sallow, wailed a couple of times, and died twenty-five minutes after birth. Both lower extremities appeared paralytic, and there was a bulge between the umbilicus and the symphysis. The testes were not palpable. — Post-mortem examination demonstrated enormously dilated ureters and distended bladder (Figs. 1 A and 1 B), and also marked bilateral hydronephrosis with large cysts on the surface. On careful incision of the urethra a valvular formation was revealed posteriorly (Fig. 1 C). Both testes were located in the abdominal cavity.

Case 2. P.M. No. 134/52. A full term male infant, three days old. Forceps delivery due to poor foetal heart sounds. The infant was languid and did not cry immediately. The abdominal wall was thin, flabby and extremely shrunken "as if the abdomen had been distended by a large tumour during foetal life". Between the umbilicus and the symphysis a cystic tumour was palpated, which varied in size and was taken to be the bladder. The intestines could be distinctly observed through the abdominal wall, which appeared to lack muscles. The testes were not palpable. The infant voided urine and took nutrition for a couple of days. Subsequently, however, it became languid and cyanotic, and died when three days old. — Post-mortem examination disclosed an extremely distended, hypertrophic bladder, dilated, tortuous ureters (Fig. 2), and bilateral hydronephrosis. Posteriorly in the urethra typical valve formation was found, while the remaining portion of the urethra was normal. The testes were located inside the abdominal wall, lateral to the distended bladder.

Case 3. P.M. No. 250/52. A male infant, ten months old, born at full term. Artificial feeding from the age of six weeks. He appeared to thrive well until the age of four

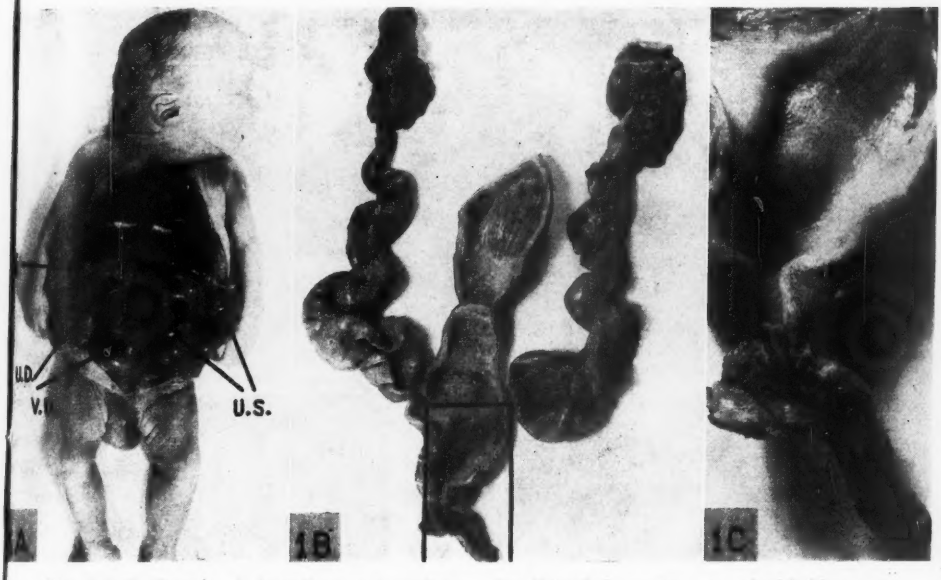
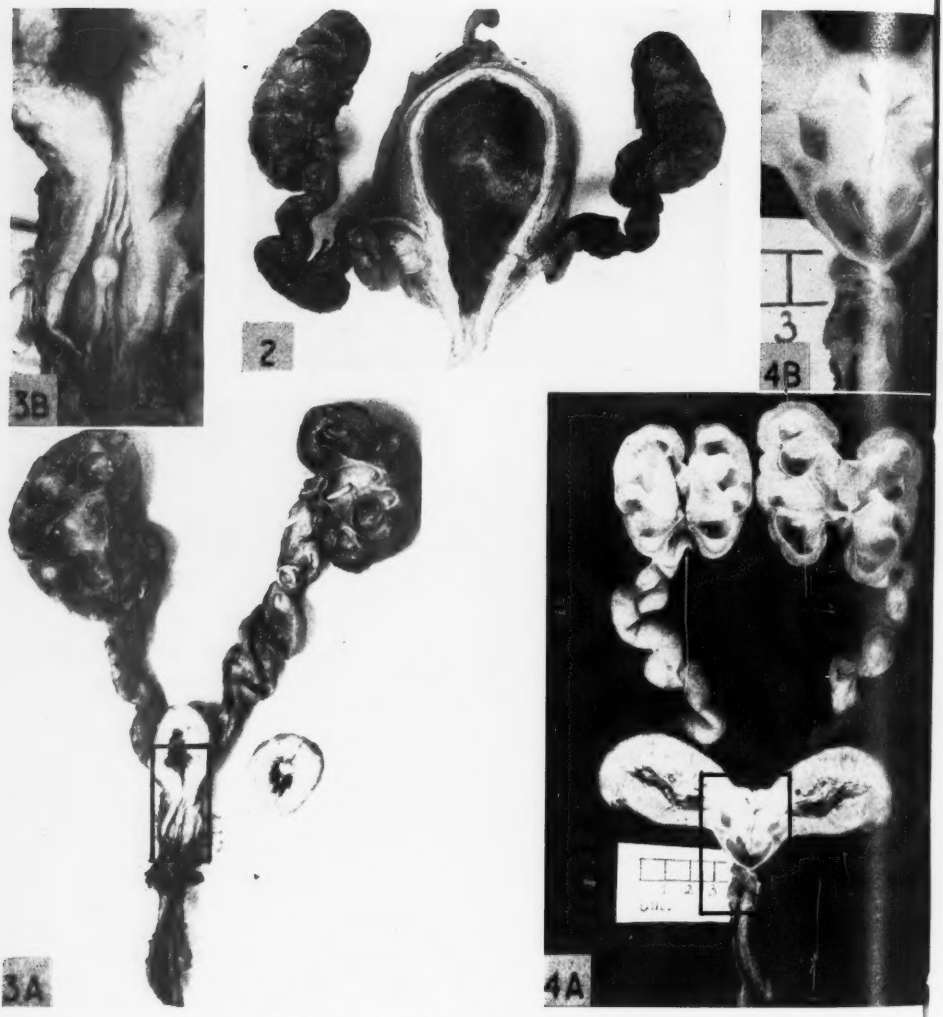


Fig. 1 A–C: Case 1. *i:* intestines. *u.d.* and *u.s.:* the distended ureters. *v.u.:* the bladder.

months, when he weighed about 7 kilos. At the age of 4 ½ months he had increasing temperature, with rhinitis and coughing. Leucocytosis was demonstrated, and the urine contained albumin and leucocytes, specific gravity 1.032. Subsequently the infant had repeated febrile periods, and was hospitalized most of the time. While the child was at home the mother noticed frequent urination, associated with distinct pain. The urine showed growth of coli and Aerobacter aerogenes, the specific gravity varying between 1.007 and 1.022. The non-protein nitrogen increased, up to 180 mg. Gradual development of a firm, persistent tumour occurred just above the symphysis, the nature of which was not definitely ascertained. The infant vomited and became more languid. In August 1952, at the age of ten months, it was admitted to the University Hospital (Rikshospitalet), Pediatric Clinic. On admission the child was considerably exhausted, with parched lips and a pitiful wailing; weight about 7 ½ kilos, i.e. about 2 kilos underweight. A firm filling, mandarin in size, was found at the site of the bladder, and on exploration a finger-thick "roll" was palpated in each lumbar region, extending downwards towards the symphysis. Urinalysis demonstrated numerous leucocytes and growth of coli and proteus. Blood urea was 83 mg, micro-sedimentation rate 22 mm in one hour. The urine came in dribbles and not in a powerful jet. The temperature remained around 38.5° C. On attempting catheterization a hindrance was encountered posteriorly in the urethra, though contrast medium passed easily, and retrograde urography showed obstructed passage in the posterior urethra, while the short upper portion of the urethra was distended. Further, "megalo-ureters" were demonstrated (probably hydronephrosis) and also diverticula in the bladder shadow.

The patient was referred to the Surgical Department, and suprapubic cystostomy



Figs. 2, 3 A–B and 4 A–B: Cases 2–4 respectively.

was performed on September 6th 1952. Despite free drainage of urine through a Pezzer catheter and administration of adequate antibiotics, the patient died nine days later. — Post-mortem examination demonstrated that both ureters and renal pelves were greatly dilated (Fig. 3 A). There was bilateral pyonephrosis with numerous abscesses in the renal parenchyma. The bladder was very hypertrophic with a lumen only the size of a hazel nut. In the posterior urethra, above which the urethra was dilated, two valves were found (Fig. 3 B). The testes were located in the scrotum.

Case 4. P.M. No. 255/52. A six-week-old male infant, born at term, had appeared perfectly healthy during the first four weeks, but had later become more languid, and during the past two weeks the appetite had been poor. During the last week he had vomited constantly, and had occasionally had hemorrhagic diarroea. On the last day he had had accelerated respiration with coughing, but no increase in temperature. On admission into the Pediatric Clinic the infant was extremely exhausted, cyanotic and dehydrated. At the site of the bladder a firm tumour was palpated, and also an irregular, soft filling deep down in the right side of the abdomen. The infant had general convulsions, and despite continuous administration of oxygen became gradually more exhausted, dying twelve hours after admission. — Postmortem examination demonstrated marked dilation of the ureter and renal pelvis on both sides (Fig. 4 A). The bladder was hypertrophic with a small irregular lumen. It was further found that the posterior portion of the urethra was enormously dilated and fibrously thickened. Probing and incision of the remainder of the urethra disclosed that it terminated posteriorly in the distended, fibrous region in such a manner that a valvular mechanism was formed here (Fig. 4 B). The testes were located in the scrotum.

Discussion

The four case reports are illustrative of how the clinical picture may vary just after birth, when the infant has to depend upon its own kidneys. Two of the infants were moribund, while the other two appeared to be perfectly healthy for sixteen and four weeks respectively, and thus showing the reserve power of the kidneys at birth.

Even though this morbid picture has been given general attention in pediatric text-books, there seems to be reason to recall the most momentous findings which may ensure an early diagnosis, thus yielding hope of effecting cure by surgical treatment: —

1. *State of urination.* Male infants—female infants are practically never victims of this malformation—void urine from the first days with a distinct jet. Intermittent seeping (Case 3), or a persistent dribble from the penis, should be a guide to the diagnosis. Sparing or strikingly irregular urination is a clinical sign which may perhaps be observed more easily by an experienced "mother of four" than in an institution in which the nursing staff works in shifts, changing several times during the twenty-four hours of day and night.

2. *Persistent tumour at the site of the bladder* (possibly also filling along the course of the ureters, as in Cases 3 and 4) indicates:

3. *Catheterization*, which is a necessary examination. It may be misleading, however: Firstly, the catheter may slip up past the valve, giving the false impression of a normal urethra. Secondly, the quantity of residual urine will vary with the degree to which the ureters are emptied into the bladder, while the catheter is indwelling. If the bladder is rigid and hypertrophic (Figs. 3 A and 4 A), the tumour above the symphysis will remain unchanged after

catheterization, and may give a false security that the tumour "is not i bladder at any rate".

These simple clinical examinations may put the investigator on the rig track soon after birth, while the recuperative power of the kidneys is st adequate. When uraemic symptoms become too dominating, associated wi possible infection, this often indicates that the possibilities of cure ha become considerably reduced. The symptoms then will become more varie and ambiguous (Cases 3 and 4).

Many of these patients die so soon after birth that there is no possibili of establishing any diagnosis, and even less of instituting any treatment. To be able to establish the exact diagnosis at all, when dilatation or other change are found in the urinary tract at post mortem examination, the urethra must always be examined *together* with the bladder. This is best achieved by remov ing the pelvic ring anteriorly before extracting all urinary tracts in one mass. Probing of the urethra takes place immediately, incision (from below) pre ferably after fixation of the dilated urinary tracts in inflated state. When the urethra has been examined *only* by probing, and has not been inspected in detail, and the hypertrophy of the bladder has not been detected, there is a risk of forming the false opinion that the striking "megalo-ureters" is the primary factor in the picture of disease.

In Cases 1 and 2 the testes were found in the abdominal cavity, before death as well as at the post-mortem examination.

Similar concurrence of anomalies has been reported in full term male infants by DALGAARD and, in twins, by DAVIDSOHN and NEWBERGER. Related findings have been made by others (1, 4, 5, 6, 9). SILVERMAN and HUANG in 1950 reviewed 18 cases from the literature and described two personal cases of (1) congenital absence (or defect) of abdominal muscles, (2) dilated urinary tract, and (3) undescended testes. They stated: "The cryptorchism is best explained on the basis of mechanical obstruction of the inguinal canal by the enormous bladder and ureters." Others also (4, 8) have explained cryptorchism as caused by mechanical blockage due to dilated urinary tract.

Whether or not the undescended testes in Cases 1 and 2 reported here are an expression of an authentic cryptorchism due to mechanical hindrance caused by the enormously dilated urinary tracts, cannot be stated definitely but the possibility of a connection cannot be refuted. It is a well-known fact that several anomalies which have no mutual correlation are found frequently in the same individual. It would seem that the movement of the testes from the abdominal cavity outwards above the pelvic brim is likely to be essentially hindered by such fillings as are shown in Figs. 1 A and 1 B.

Descent of the testes usually takes place in the eighth month of foetal life. Absence of descent should give rise to the suspicion that dilatation of the

urinary tract, and consequently the renal damage, have started relatively early in foetal life, and this circumstance, therefore, should be interpreted as a sign of poor prognosis.

Summary

Four fatal cases of congenital valvular formation of the posterior urethra in boys are reported, two of which presented greatly dilated urinary tract at birth, and simultaneously cryptorchism.

Formation valvulaire congénitale de l'urètre postérieur.

4 cas mortels de formation valvulaire congénitale de l'urètre postérieur survenant chez des enfants sont rapportés. Deux d'entr'eux présentaient une importante dilatation du tractus urinaire, avec cryptorchidie concomitante.

Kongenitale Klappenbildung der hinteren Urethra.

Es wird über 4 tödlich verlaufene Fälle von kongenitaler Klappenbildung in der pars posterior der Urethra bei Knaben berichtet. In zwei dieser Fälle fand man bei der Geburt stark erweiterte Harnwege mit gleichzeitigem Krytorchismus.

Formación valvular congénita en la uretra posterior.

Se comunican 4 casos fatales de formación valvular congénita en la uretra posterior. Dos de los niños presentaban criptorquidia y gran dilatación de las vías urinarias al momento de nacer.

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CASE REPORT

Idiopathic Renal Acidosis

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Idiopathic renal acidosis (Lightwood-Albright's Syndrome) is a disease only recognized in the last two decades. It appears to occur most frequently in infants but has been diagnosed also in older children and adults. It is characterized clinically by anorexia, decreasing well-being, periods of vomiting, a tendency to dehydration and constipation.

The blood findings are a markedly reduced alkaline reserve and hyperchloremia, normal urea and non-protein N. In spite of the acidosis the urine has an alkaline or slightly acid reaction. This contradictory finding is the most important criterion of the disease (3). Usually there is isosthenuria or hyposthenuria, but normal specific gravity has also been observed (3). Protein reactions are slightly positive or negative. Microscopy may show small or negligible amounts of solid elements. The urine is sterile and urography usually normal. In some cases considerable calcium deposits have been found in the kidneys, and LIGHTWOOD (9), who was the first to describe the disease, called it nephrocalcinosis infantum after the most important symptom in his case. Subsequent experience has shown that calcium deposits are not always present and that they are also found in other kidney diseases. In idiopathic renal acidosis roentgenologically demonstrable calcifications are usually a late symptom, but before they appear the section has shown extensive calcifications around the renal tubuli, particularly in the deeper parts of the medulla (3).

Pathologic study reveals kidneys macroscopically normal and without anomalies of the kidney pelvis or urinary canals. Microscopic examination shows normal glomeruli, but in several cases marked changes were seen in the tubuli where thickening of the basal membrane and atrophic cells appeared. However, the findings are not uniform on the latter point. There is a uniform absence of gross changes of the type caused by nephritis or arteriosclerosis (3, 2, 6). Pathologic findings in other organs were absent except for extensive calcifications in a few cases.

The prognosis generally has been considered poor, even though the course of the disease may vary. However, several cases have recently been reported (3) in which an early diagnosis was made and complete cure appears to have been effected after protracted treatment with alkalies. Successful outcome has been reported by others (13, 7) in isolated cases. Since there is still only a small number of cases on record and only a few of these have been followed for an appreciable period of time, it is difficult at the present time to form a definite opinion as to the prognosis. There are also several other unclarified points in the disease picture, and therefore the following case may be of interest. (Similar cases have apparently not been published from Scandinavia.)

Female, born Feb. 14, 1951. (Serial No. 13833/52.) Parents and 1 brother, aged 4, living and well. No known kidney disease in the family. Pregnancy and delivery uncomplicated. Birth weight 3,000 g, height 51 cm. The infant was well during the neonatal period. Breast feeding was given for 2½ months, after which a 2/3 cow's milk mixture was used. After the first few weeks the infant showed a tendency to regurgitation which progressed to vomiting when artificial feedings were started. Appetite was not good and weight gain unsatisfactory. Weight at 4 months was 4,100 g. Violent vomiting with fever began in the beginning of June 1951, and the infant was admitted to the local hospital. Penicillin treatment and subcutaneous normal saline solution were given, but the fever remained high, 39–40° C, and the vomiting continued. After 9 days of acute illness she was transferred to the Children's Department, University Hospital, Oslo. The findings on admission June 12, 1951, were: pallor, muscular hypotonus and reduced tissue turgor, sunken fontanelle and dry tongue. Temperature was 40.5° C. Otoscopy revealed injection and slight bulging of the left eardrum. Paracentesis was performed. No drainage followed. No other pathological findings were made at the general examination. Wt. 3,940 g, height 68 cm. Urinalysis: Spec. gravity 1,010, protein present, microscopic: very few leukocytes. Hb 100 %, rbc: 4,640,000, wbc: 37,200 with shift to the left. Plasma bicarbonate 25 vol. %.

Penicillin treatment was given for 4 days followed by streptomycin for 1 week during which the temperature returned to normal. Ringer's and glucose solution were given parenterally, and transient edema was observed. Vomiting ceased at the time of admission but occurred sporadically later.

The plasma bicarbonate remained low, 25–27 vol. % seen in repeated tests, urea 30 mg %, chlorides 122 m. Eq./l. Spec. gravity of the urine remained low, under 1,012. Slight positive protein reactions or a few leukocytes were found in a few of the urine examinations. Others were negative. Urine cultures were negative.

Sodium bicarbonate treatment using a dose of 1–2 g daily resulted in a rise in alkali reserve to normal levels parallel with a gain in weight. Discontinuation of the medication caused immediate return to the earlier values. Continuous doses appeared to keep the level within normal limits, and the child was sent home on a maintenance dose of 1 g sodium bicarbonate daily.

She has since been admitted to the department 3 times at about half-yearly intervals. Her condition at home seemed good, and her parents therefore gave her the medication only for short periods during the first year when vomiting occurred. However, her appetite was only fair and constipation constant. She had a tendency to vomit, especially during acute infections. From Dec. 1952 to April 1953, she took a mixture of 3 g sodium and 1 g potassium citrate daily. She thrived much better on this treatment, her appetite increased and constipation decreased. Her height continued under the average for her age. On admission Apr. 22 1953 her height was 81 cm (average 89 cm), wt. 10 kg. Her mental age was quite normal for her chronological age.

The following examinations were made during her 3 subsequent hospitalizations (the results varied only slightly from one hospitalization to the next): *Blood pressure* remained normal at about 100 systolic and 60 diastolic. *Urine:* pH varied between 6.5 and 6.9, usually 6.8 (electrometric measurements). Specific gravity remained almost constant at 1.010 to 1.011. Dilution and concentration tests showed 1,005 and 1,024 as minimum and maximum values. Protein reactions were negative or slightly positive. Microscopic examinations showed a few white blood cells occasionally.

TABLE 1

Biochemical findings and treatment.

Date	Medication in g	Plasma-bicarb. vol. %	Serum-chloride m.Eq/l.	Total base m.Eq/l.	Urine bi... vol.
17. 7.51		25	122		
7. 3.52		22	119	156	
20.10.52		20	125	141	
11.11.52		27	116	142	6
15.11.52	Sod.cit. 3, Pot.cit. 1				
17.11.52		46			29
19.11.52	Sod.cit. 4, Pot.cit. 0.3				
26.11.52		38	111		29
27.11.52	Sod.cit. 2, Pot.cit. 0.6				
1.12.52		40	106	145	42
2.12.52	Sod.cit. 3, Pot.cit. 1				
8.12.52		54	109	147	91
22. 4.53		29	115	146	
29. 4.53		26	117	150	9
2. 5.53	Sod.chlor 3				
4. 5.53		25	128		9.9
5. 5.53	Sod.cit. 3, Pot.cit. 1				
8. 5.53		31	103		14
	Sod.cit. 6, Pot.cit. 2				
11. 5.53		48	103	148	57.4
13. 5.53		43	114	154	48

Cultures were negative. Bicarbonate in the urine was 5–10 vol.% with marked increase during alkali medication (see Table). Ammonia excretion (formalin titration) approximately 90 mg/24 hours. Free acid by titration to pH 7.4 was 2–3 m. Eq./l. Sulkowitch reaction: slightly positive, more strongly positive at the last examination (after discontinuation of the alkali medication). Glucose tolerance (5 g/kg): no sugar excretion, blood sugar curve normal. Paper chromatography Apr. 1952: no pathological amino acids. Diuresis (without alkali treatment) varied between 500 and 720 ml/24 hours.

Blood: The amount of bicarbonate in the plasma remained low in the absence of alkali medication. On admission Apr. 22, 1953, it was low in spite of a reported regular use of alkali in the dose which had been sufficient to correct the acidosis 6 months previously. Serum chlorides, usually somewhat high, rose appreciably after administration of 3 g NaCl daily (see Table). Urea: 18–35 mg%. Blood creatinine: 0.5–0.9 mg%. Standard urea clearance (corrected to 1.73 m^2 excess): 23–28 ml/min. Creatinine clearance (corrected): 54 ml/min. Creatinine tolerance test: 42–46% of the expected normal excretion. Calcium: 10.4–10.8 mg%. Phosphorus: 4.3–5.1 mg%. Phosphatase: 7.2–13.9 units. Blood counts: nothing abnormal. Sed. rate: normal. Plasma prote...

Albumin 5.05–5.23, Globulin 2.41–2.47. Bone marrow puncture (Apr. 1952): nothing abnormal.

Roentgen examinations: The skeletal system showed slight osteoporosis: no signs of rickets. Intravenous urography: Comparatively good excretion: Somewhat distended calyces on both sides. Otherwise normal. Retrograde urography (rt. side): nothing abnormal. During the last hospitalization (Apr. 1953) 2 small calcifications were seen in the left kidney for the first time. Cystoscopy: normal.

Results of treatment: The changes in the blood electrolytes and bicarbonate excretion may be seen in the Table. In addition to the satisfactory symptomatic effect shown by reduced thirst and diuresis there was steady gain in weight. Discontinuation of the medication during the last hospitalization produced loss of weight of 0.9 kg in 10 days. This was promptly regained after treatment was resumed. However, increasingly larger amounts of alkali were necessary to maintain normal bicarbonate values in the plasma, i.e., 1–2 g, 4 g, and 8 g respectively during the last 3 periods of hospitalization.

Discussion

As mentioned previously it is the abnormal relationship between the severe acidosis and the slightly acid or alkaline urine which is outstanding in the disease picture and of primary importance in the diagnosis. Ordinarily in acidotic conditions all bicarbonate is absorbed in the tubuli, and in adults it is not excreted in the urine until the alkali reserve is above 26–28 mmol, the equivalent of 58–62 vol.% (11). In contrast, alkali is excreted in the condition studied here in the presence of marked acidosis.

Regarding the pathogenesis of the disease it was at first thought by ALBRIGHT and his co-workers (1) that a loss of the kidney's ability to eliminate acids or to form ammonia was the cause. Tests (8) in patients suffering from the disease were made using an infusion of a mixture of primary and secondary sodium phosphate. The tests showed that this ability was normal. The theory which is now apparently accepted by the majority is that a reduced ability to reabsorb bicarbonate in the proximal part of the tubuli causes the condition.

The reduced production of ammonia found in some cases is thought to be secondary, caused by the increased amounts of bicarbonate in the distal parts of the tubuli.

The disease has its major effect upon the tubuli. However, it appears that the glomeruli may also be affected, perhaps secondarily. The reduced values for urea and creatinine clearance in the present case indicate such damage.

The actual cause of the decreased reabsorption of bicarbonate is not known. Toxic damage to the cells of the tubuli following sulfonamide medication has been suggested as a cause (4, 5). This assumption could be supported in part by PITT's theory (10) which states that carbonic anhydrase is an active ferment in the reabsorption of bicarbonate. Carbonic anhydrase is hindered in its function by sulphanilamide, and acidosis with alkaline urine may occur as a result of its use. However, it appears that this effect arises from damage to the cells in the *distal* part of the tubuli where only a small part of the reabsorption of bicarbonate is assumed to take place. In most cases, the present case included, there is no history of the use of sulfonamides. A congenital dysfunction of this enzyme might be followed by a decreasing HCl production in the stomach. Examination of the stomach secretion after histamine injection showed normal amounts of acid in this patient.

A congenital anomaly seems to be the most reasonable cause of the disease as it developed in this case. The symptoms were fully developed at the age of 4 months, but seemingly they began in the earlier months when the infant showed signs of poor appetite, vomiting and retarded weight gain. It is possible that the condition is an isolated case of dysfunction of the tubuli in the same category as renal glycosuria, de Toni-Fanconi syndrome and phosphodiabetes (FANCONI).

Signs of dysfunction in other organs besides the kidneys could not be found.

Among the biochemical findings hyperchloremia was considered compensatory to the reduced plasma bicarbonate. When supplementary doses of sodium chloride were administered there was a marked rise in serum chlorides (see Table). This condition has been observed by others (1). The disturbance of the chloride excretion may be considered a result of the loss of the normal reabsorption mechanism for bicarbonate. According to PITTS and co-workers (11) this is a selective process. If reabsorption of these anions occurs passively in connection with the reabsorption of sodium, one may assume that Cl passes more quickly and easily back from the tubules than HCO_3. Furthermore it appears that the excretion of these anions is regulated so that an excess of chlorides causes a reduction of the reabsorption of bicarbonate (12). This was not particularly noticeable during sodium chloride medication in this patient. Marked increase of the clinical symptoms after dosage with sodium chloride has also been observed (3). This condition may be assumed to be accentuated by reduced filtration, even though hypochloremia is commonly found in chronic glomerulo-nephritis. A moderate intake of salt would therefore appear to be advantageous in idiopathic renal acidosis.

Calcifications in the kidney region were first seen in this patient after the disease had been recognized for nearly 2 years and after continuous alkali treatment had been given for 5 months previous to the findings. Alkaline urine has been considered the most important predisposing factor for calcification. However, calcifications are a common though late symptom in these patients, even those not treated. It is not likely that the reaction of the urine is the decisive factor, since urine with a pH of approximately 7 is not absolutely non-physiologic and can be produced by the use of a predominantly lacto-vegetable diet.

Abnormal excretion of calcium in the urine was not observed in this patient. Sulkowitch reaction was very slightly positive for a long period but became somewhat more strongly positive, though not pathologic, during the last hospitalization. The calcium and phosphorus values in the blood remained normal. There was only a slight osteoporosis, and no sign of rickets. The chemical changes of the blood and urine do not provide a completely satisfactory explanation for the pathogenesis of the calcifications. It is more likely that they are a direct result of the pathologic cell activity in the tubuli.

The demonstration of calcifications makes the prognosis doubtful. There was also a marked increase in the amount of alkali needed. Other signs of progression were not found. The general condition was markedly improved when the alkali treatment was given continuously, though the tendency to acidosis remained present. Whether a permanent cure would have resulted if the parents had followed instructions for treatment from the outset is impossible to determine. Several of the cases (3) reported as completely cured had a normal specific gravity of the urine and differed in other respects from this and other cases. The differences may depend on a varying degree of the severity of the anomaly, but there is also the possibility that they are pathologically different diseases with similar symptomatology.

Summary

A case of chronic acidosis diagnosed at the age of 4 months and followed for 2 years is discussed. The biochemical changes in the blood and urine were similar to those described in idiopathic renal acidosis. Calcifications in the renal parenchyma were first demonstrated by roentgen examination at the age of 2 years. There was no etiological explanation for the patient's condition which, being apparent from the first months of life, appeared to be congenital. Alkali treatment had a beneficial effect on the symptomatology but no curative effect upon the acidosis.

L'acidose rénale idiopathique.

Un cas d'acidose chronique, diagnostiqué à l'âge de 4 mois et suivi pendant 2 ans, est discuté. Les modifications biochimiques du sang et des urines sont semblables à celles décrites dans l'acidose rénale idiopathique. Des calcifications dans le parenchyme rénal ont été mises en évidence, par l'examen radiologique, pour la première fois, à l'âge de 2 ans. Il est difficile de fournir une explanation étiologique; le début des symptômes, dans les premiers mois, parait en faveur d'une origine congénitale. Un traitement alcalinisant a eu un effet favorable sur la symptomatologie, mais sans guérison de l'acidose.

Idiopathische renale Acidose.

Es wird ein Fall von chronischer Acidose besprochen, der mit 4 Monaten diagnostiziert und über 2 Jahre lang verfolgt wurde. Die biochemischen Veränderungen im Blut und Urin waren den bei idiopathischer renaler Acidose beschriebenen ähnlich. Verkalkungen im Nierenparenchym wurden im Alter von 2 Jahren zum erstenmal roentgenologisch festgestellt. Dieser seit den ersten Lebensmonaten bestehende Zustand des Patienten konnte ätiologisch im Sinne einer kongenitalen Erkrankung gedeutet werden. Alkalitherapie hatte ganz gute symptomatische Wirkung, aber die Acidose selbst wurde nicht behoben.

Acidosis renal idiopática.

Se discute un caso de acidosis crónica, diagnosticado a la edad de dos años. Las modificaciones bioquímicas de la sangre y orina son similares a las descriptas en la acidosis renal idiopática. Se han visto calcificaciones en el parénquima renal (por medio del examen radiológico) por primera vez a la edad de dos años. Es difícil dar una explicación etiológica; el comienzo de los síntomas, en los primeros meses está a favor de un origin congénital. Un tratamiento alcalinizante ha tenido un efecto favorable sobre la sintomatología pero sin curación de la acidosis.

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The Danish Paediatric Society

Meeting, November 4, 1953.

K. Seidel: **Discussion on the arrangement of certified milk for children and infants (so-called "Children's Milk").**

A historical review of the conception of "Children's Milk" was given. The sale of "Children's Milk" in Copenhagen started in about 1880, but at that time no laws or regulations concerning the hygiene of milk were in existence. The idea of "Children's Milk" occurs for the first time in an addendum to the Public Health Regulations, May 3rd 1904, where it is in fact defined in the same way as in the regulations which appertain today. An account was then given of the various detailed regulations which appeared in the Public Health Regulations and the Milk Laws on this subject. The modern requirements for Children's Milk were also mentioned. Agreement between dairies and producers of Children's Milk has been reached on additional payment for milk with a particularly low content of micro-organisms. The retail price of Children's Milk in November 1953 was 63 öre per litre and for ordinary milk 51 öre per litre. The dairies are classified by the Milk Marketing Board according to the hygienic quality of the various types of milk that they have produced in the previous month. The hygienic standards of both Children's Milk and of ordinary milk have been raised during the 3 years this system has been in use. These results were expressed by tables.

The consumption of Children's Milk has decreased and the supposed causes of this decrease were discussed. Four possibilities concerning the future sale of Children's Milk and similar products were suggested: (1) Maintenance of the present Children's Milk arrangement, by which Children's Milk of high hygienic standard is sold in the raw state. (2) Maintenance of the Children's Milk arrangement as mentioned under (1), with the additional provision that Children's Milk in future undergoes treatment with heat (low pasteurization or stassanization). (3) Discarding the conception "Children's Milk". (4) Sale of a type of milk, labelled for example A-milk, as a substitute for the present Children's Milk.

The question of homogenization and vitaminization of the suggested so-called A-milk was mentioned.

Discussion. *Ester Ammundsen.* By introduction of a new milk statute for Copenhagen it has been considered necessary to obtain information whether the paediatricians still desire to maintain the arrangement for Children's Milk as it exists at present, or possibly including treatment with heat. It seems to be an unanimous feeling that the present arrangement with raw milk should be discarded. Pasteurization would further increase the price of Children's Milk. If the paediatricians insist upon strict hygienic precautions when the milk is pasteurized, a possible solution would be to produce a low-fat (2.5 %) pasteurized Children's Milk. In this way, the price would be reduced, and consumption could be limited to infants as this milk would not tempt the upper

classes. Also the low fat percentage would be more suitable for infants. — *Oluf Anders* . Experience shows that milk, cautiously treated with heat, may be employed with detriment both to healthy and sick infants and that the requirement that all Childre Milk must be raw cannot therefore be maintained. It appears, in addition, that high fat content in the milk is badly tolerated by infants and it would therefore appropriate to standardize Children's Milk at a low fat percentage. The requirement that the milk must have a low content of micro-organisms must, on the other hand, be maintained. Strongly polluted milk may, even when sterilized, contain a danger because certain bacteria contain thermostable endotoxins which may cause digestive disturbances in infants. It would be desirable that the dairies deliver the milk in containers, safely sterilized. An arrangement was recommended according to which Children's Milk of low micro-organism content from controlled stocks be maintained; that this milk should be standardized to a lower fat content, e.g. 2.5 per cent, and that the milk be treated with heat. — *C. Mollenbach.* Feeding experiments could not demonstrate any difference in the tolerance of infants to the milk from cows fed on silage and draff as compared with ordinary Children's Milk. — *P. Bræstrup:* Reduction of the fat percentage is advantageous both nutritious-hygienically and economically. No criteria are available that any difference exists between milk —raw, heat-treated, homogenized or vitaminized or as dried milk —as regards nutrition. The consumption of milk in Copenhagen is low compared with the consumption in Oslo and Stockholm and efforts to increase the milk consumption seem to be indicated. A heat-treated, low-fat (standardized) Children's Milk was proposed. — *K. Biering-Sorensen:* According to their instruction, the Health Visitors inform the mothers that ordinary milk is just as good as Children's Milk. More than half of the Health Visitors state that only 0–5 per cent of the infants under their care receive Children's Milk. Biering-Sorensen had received the impression from the Health Visitors that ordinary milk is tolerated better than Children's Milk. — *A. Rothe-Meyer* was of the opinion that the standard of the retail milk in Copenhagen is not optimal. Desired a heat-treated milk, standardized at a low fat content and of a guaranteed satisfactory hygienic quality. — *P. Druckerr* had previously, in the Medical Association's Committee of Hygienics, advocated raw Children's Milk but had now changed his standpoint and recommended heat-treated, low-fat Children's Milk. — *H. O. Pedersen.* Raw Children's Milk, as produced now, is innocuous for infants. Three types of milk were proposed: (1) A type, poor in micro-organisms, treated with heat and with low fat content for infants. This milk ought to originate from recognized and controlled stocks. (2) Children's Milk maintained raw as previously. (3) Retail milk as previously. — *Urban Hjärre* expressed his unreserved recognition of milk control in Copenhagen and regretted that the quality of milk in Stockholm was poor, measured by Danish standards. Informed the Delegates that the content of micro-organisms in milk in Stockholm had increased catastrophically after the control had become less strict. The maintenance of controlled milk was recommended, but as regards the question of heat treatment, the problem should be studied whether raw milk contains bactericidal substances which are destroyed by heat treatment. — *F. Tudvad.* What will be the fate of Children's Milk outside Copenhagen if the Children's Milk in Copenhagen is pasteurized? The production of Children's Milk should be centralized in a single dairy. This would involve greater possibilities for research and would facilitate co-operation between dairy personnel, veterinary surgeons and physicians in solving some of the less well-elucidated problems, e.g. the optimal fat percentage, the homogenization and vitaminization. Doubted whether a price differen-

of 12–13 öre per litre is decisive if really recommendable milk is obtained. — *A. Rothe-Meyer* supported the proposal of centralization of the production of Children's Milk and mentioned the significance of the treatment of retail milk after pasteurization. Proposed that the date stamping be extended to morning and evening stampings. — *E. Ammundsen* regarded it as necessary that if Children's Milk be introduced in an altered form, the price must not exceed that of retail milk. — *E. Wamberg* mentioned experiments by himself and others with homogenized milk. Normal children and infants may absorb 97 per cent of the fat in ordinary milk. In children with dyspepsia, the absorption of fat may only be improved in isolated cases by the administration of homogenized milk. Neither does homogenized milk offer any advantages to premature infants. By and large, homogenized milk offers no nutritional advantages. — *K. Seidel* stated that 80–90 per cent of the bottles are sterile. The introduction of waxed containers, as proposed, would, according to available information, increase the expense by about 10 öre per unit. The reduction of the fat percentage in Children's Milk to 2.5 per cent would approximately cover the expenses for heat treatment. Centralization of the production of Children's Milk is a good idea but difficult to put into practice. — *O. Winther.* The bactericidal substances in milk are only slightly influenced by low pasteurization while high pasteurization will cause the milk to deteriorate rapidly. — *Sv. Heinild* proposed that 3 types of milk be produced: (1) Dried milk for infants; (2) Retail milk as previously; (3) Raw Children's Milk.

Meeting, November 23, 1953

Esther Elkjær-Laursen and *J. Flamand-Christensen:* **Investigation into the growth and development of premature infants in the town of Esbjerg.**

Based on midwives' records, the mortality among premature infants in Esbjerg during the period 1944–50 (a total of 436 infants) was investigated. A relatively large mortality was found, particularly among infants of birth weights between 1,000 g and 2,000 g. Based on health visitors' records, the methods of feeding, thriving, growth, degree of development and morbidity in the same group of infants during the first year of life was investigated. Ninety-three died during the first month of life and before supervision was established. A total of 288 infants was followed up for more than 9 months; this group comprises 4 infants with birth weights ≤ 1,500 g, 45 with birth weights 1,501—2,000 g, and 239 with birth weights 2,001—2,500 g. One hundred and sixty-three infants were born and looked after at home while 125 were born in hospital or admitted to hospital after delivery. The incidence of breast-feeding fell only slightly below that of infants in general. The stay in hospital did not involve any lasting compromising of breast-feeding. A comparison between the growth of artificially fed and breast-fed infants shows that breast-fed infants increased most in weight during the first 3 months of life while the artificially fed infants grew better than did the breast-fed infants in the second trimester (the difference is, however, only significant in the group 2,001—2,500 g from the 4th to the 6th month). The growth was independent of the mother's receptiveness to advice and independent of housing conditions. The somatic and psychomotor development of the infants at the age of one year approximated, in the majority of cases, to normal. The morbidity was the same in premature infants and in infants of normal birth weight. The morbidity in breast-fed infants was found to be half that encountered in artificially fed infants and, similarly, the morbidity under good housing conditions

was only half that encountered in infants living under poor housing conditions. The mortality was, however, approximately thrice the normal in the ages 2–12 months.

The main impression created by the investigation is that premature infants, on the whole, get along well in the home environment and that infants between 1,501 g and 2,000 g did not thrive worse than did the larger infants. It will therefore, in general, be justifiable to entrust the care of the infant to the mother and the health visitor as soon as the infant has begun to thrive and shows normal physiological reactions without adhering to any particular weight limits. Also from a psychological point of view, it is undoubtedly advantageous that too long a period does not elapse after the delivery until the mother takes over the care of her infant. If more favourable results are to be achieved in the treatment of premature infants, the main object must be to combat the mortality associated with the delivery (a task for the physician and the hospital) and the morbidity during the first year of life. Improved breast-feeding would be of significance, but the decisive factors are social and improvement of housing conditions is undoubtedly the most important task.

Discussion. *P. Bræstrup* was pleasantly surprised that so much information could be deduced from the health visitors' records but doubted if it was justifiable to stress the significance of breast-milk for premature infants and recommended rather the reinforcement of breast-milk with protein, calcium etc., not only in hospital wards but also in the home. — *A. Rothe-Meyer* remarked that the calculations of the percentages were based on widely varying figures. — *J. Flamand-Christensen* admitted that the material was too limited, but it had been impossible to increase it under the given conditions as it represents the population of the town of Esbjerg. In addition, a control material of infants, not supervised by health visitors, is lacking.

Peer Pørregard: **Blood in the faeces of premature infants. A sequel of iron medication?**

Fresh blood in the faeces was observed as the only sign in a total of 32 out of 203 hospitalized premature infants, treated with iron. The sign occurred most frequently in infants with a birth weight of under 2,000 g and most frequently in the second and third week of medication with iron. From June 1951, iron was systematically withdrawn when fresh blood appeared in the faeces. The blood then always disappeared in the course of 24 hours. When the faeces had become normal again, iron therapy was recommenced. Previously, on numerous occasions, blood was observed in the faeces for 12 days in succession and on one occasion for as long as 29 days. From June 1951 until the end of 1952, 24 infants showed blood in the faeces. Of these, 19 received iron on a second occasion, whereupon 10 of them again showed blood in the faeces; 5 infants received iron on a third occasion and 3 of them showed blood in the faeces again. One infant received iron on a fourth occasion, whereupon blood appeared for a fourth time. The diet received by these infants was the diet usually employed in departments for premature infants, including a supplement of vitamins A and D and ascorbic acid but otherwise with no drugs apart from iron. The faeces were completely normal. Anal inspection and rectal exploration were carried out on numerous occasions and revealed nothing abnormal. Proctoscopy was performed on an isolated occasion and showed redness and swelling of the mucous membrane. The present material does not permit of any definite conclusions but it seems to indicate a certain connection between the isolated occurrence of blood in the faeces and iron medication in premature infants.

Discussion. *B. Bærstrup:* Has the possibility of a defective iron preparation (e.g. containing ferric compounds) been considered? — *P. Pærregård:* No, but the distribution of the cases was uniform during the whole period of investigation and the supply of iron powder was renewed several times. — *Sv. Brandt:* Were the infants constipated? — *P. Pærregård:* No. — *A. Rothe-Meyer:* Was there only fresh blood in the faeces? — *P. Pærregård:* Yes, only small streaks of blood in the faeces. — *H. Friis-Hansen:* Did similar signs occur in infants which did not receive iron? — *P. Pærregård:* No. — *J. Biering:* In what chemical form did the iron appear in the faeces? — *P. Pærregård:* This was not investigated.

Ib Munkvad and *Jørgen Vesterdal:* **Plasma glutamine and glutamic acid in normal children and in children with cerebral affections.**

Determinations of glutamine and glutamic acid in the plasma by Krebs' method were performed in 12 children without and in 25 children with affections of the brain, particularly Little's disease and epilepsy, and finally in 5 children suffering from neurosis. Highly varying values were found in the same child at different times. Children suffering from affections of the brain showed the same concentration of glutamic acid in the plasma as did the children in the control series but their concentration of glutamine was lower. The test is of no diagnostic value. Further investigations on this subject are desirable.

Discussion: *Sv. Brandt:* Was any difference found between children with epilepsy and children suffering from other affections of the brain? — *J. Vesterdal:* No.

Meeting, December 2, 1953

Olaf Andersen: **Endocrinology in childhood.**

Henning Andersen: **Cortisone therapy of the adreno-genital syndrome.**

Case 1. A girl, now aged 16 years, with congenital hyperplasia of both suprarenal glands, confirmed histologically. During continuous cortisone therapy over 2 ½ years, menstruation occurred as well as complete development of the female secondary sex characteristics. The hirsuitism, masculine voice and muscular bodily configuration disappeared. The clitoris decreased in size by approximately 1 cm. On withdrawal of the drug for 3 weeks after 2 years therapy, increase in the excretion of 17-ketosteroids of approximately ¼ of that prior to therapy occurred while, on the other hand, the excretion of cortisone in the urine increased quite considerably. The treatment had no undesirable effects.

Case 2. A girl, aged about 8 years, with congenital adreno-genital syndrome and agenesis of the right kidney and ureter. Explorative operation about 1 ½ years ago showed slightly hyperplastic suprarenal glands on both sides. Since then treated with cortisone. Her growth and development have thereafter followed her skeletal age. The virilism is diminishing. Withdrawal of cortisone for some weeks after over a year's continuous treatment gave a result corresponding to that in the first patient. The therapy had no undesirable effects.

Case 3. Girl, aged 4 years. During the preceding months, slight hypertrophy of the clitoris and development of pubic hair. The late appearance of the symptoms and

the fact that the patient was of less than average height for her age, aroused the suspicion of suprarenal tumour. The 17-ketosteroids were excreted in the urine in two to thrice the normal quantity and remained at this level during intramuscular treatment with cortisone. These findings were considered strong evidence that a tumour was concerned and not hyperplasia of the cortex. The final diagnosis was established on tomography after administration of 50 per cent Diodone. This examination revealed a shadow as large as a half crown at the site of the right suprarenal gland. At operation shortly afterwards, a benign adenoma of the right suprarenal cortex was encountered. Thereafter, the excretion of 17-ketosteroids fell to normal levels.

Conclusion: Cortisone, at the moment, is the drug of choice in the treatment of virilism as a result of hyperplasia of the suprarenal cortex and an important aid in the diagnosis of tumour of the suprarenal cortex from hyperplasia.

(Will be published in detail later in "Nordisk Medicin".)

Discussion: *Chr. Hansted* reported briefly the results of this treatment in 4 patients with the adreno-genital syndrome. — *Chr. Hamburger:* Attention was drawn to the recent investigations by the Canadian anatomist Dr. Murray L. Barr concerning the so-called "Sex Chromatin" which appears in the cell nuclei of the majority of cells in the female but which only seldom can be demonstrated in the cells of the male. By simple microscopical examination of tissue, e.g. from a skin biopsy, stained in the usual way it may be determined with certainty whether the tissue originates from a male or from a female. The occurrence of "Sex Chromatin" is supposed to be connected with the presence of 2 X-chromosomes and is only rarely encountered where X — Y-chromosomes are present. Thanks to Barr's investigations it seems to be possible to determine an individual's chromosomic or genetic sex in a very convenient manner.

Flemming Quaade and *Henning Andersen:* **The effect of d-Amphetamine on adipose children.**

One hundred and three adipose children from 4 to 16 years were treated as out-patients in the clinic for endocrine diseases in Queen Louise's Hospital for Children. Alternate children received d-amphetamine and the others a placebo, both substances being administered in doses according to the body weight (up to 32.5 mg/kg/day). The children were instructed not to partake of any special diet. Thirty-three children, mainly from the placebo group, had to be excluded from the series for various reasons. The remainder, 25 boys and 45 girls, received tablets continuously for periods varying between 1 and 11 months. The sole object of the investigation was to decide whether d-amphetamine was capable of producing a loss in weight in adipose children. The analysis reveals that while the children from the placebo group increased somewhat in weight during the experimental period, a marked fall in weight occurred in the group treated with d-amphetamine, and in the majority of the individual curves a definite agreement is noticeable between loss in weight and medication with d-amphetamine. Nearly all the children stated that their appetites diminished greatly when they consumed the d-amphetamine tablets. Side-effects occurred in 20 out of the 70 children, most frequently taking the form of difficulty in falling asleep at night, but these undesirable symptoms always disappeared rapidly and in no case necessitated reduction of the dose or withdrawal of the drug.

Discussion: *Johanne Christiansen:* Children treated for overweight have their consumption of milk reduced to half a litre or less but have frequently not been forbidden sugar and sweets nor white flour and margarine. A prophylactically effective propaganda from the organization of paediatricians similar to that of Toverud and Carl Schiötz in Norway is lacking. Nodaways sweets and sugar are forbidden in several schools in Norway and food brought from home is under the control of the school. The Oslo Breakfast is much more rational than are the Danish school lunches. The advisory propaganda issued by the Bureau for Housekeeping leaves much to be desired. It is a poor substitute for the schemata issued by the League of Nations, recognized by all authorities on nutrition, in which sugar, white flour and margarine are excluded from the diet of children and one litre of milk plus one egg daily are recommended. A country where bad teeth and many degenerative diseases are encountered needs ideals, not compromises, as guidance for the population. — *J. Flamand-Christensen* mentioned the possibility that the fat children treated with d-amphetamine receive an even more faulty diet than they did prior to treatment. — *P. Plum* was of the opinion that it is possible to some degree to alter the dietary habits of fat children and that the reducing diet should not be completely abandoned. — *P. Bræstrup* found the loss of weight modest in comparison with that which may be achieved with a reducing diet. — *Fl. Quaade.* Agreed with Johanne Christensen and was of the opinion that extended instruction on nutritional hygiene is necessary. He had experienced no favourable results from attempts to treat an out-patient clientele with reducing diets.

Erik Ryssing: **Investigation concerning the effect of phyol.**

Phyol (a proprietary preparation from Alfred Benzon Ltd., containing the pituitary growth promoting principle) in doses up to 10 ml administered to 2 children without pathological conditions of the hypophysis, exerted none of the characteristic effects of the growth hormone on the urinary excretion of phosphorus, urea, nitrogen, potassium, sodium or chlorides. On the other hand, Phyol contains some A.C.T.H. which may camouflage a possible growth hormone effect. Phyol does not influence the carbohydrate metabolism, not even during intense A.C.T.H. treatment.

(Will be published in "Ugeskrift for Læger".)

Discussion: *Chr. Hamburger:* What is the proportion between the doses for rats and man? — *E. Ryssing:* Difficult to compare. — *Henning Andersen:* Phyol may contain thyreotropin in addition to A.C.T.H.; investigations on mucopolysaccharides in the skin indicate this. — *R. Wichmann* found in 3 preparations of Phyol sufficient thyreotropin to be biologically active.

Henning Andersen: **The diagnosis of hypothyroidism in children.**

A case of simultaneous agenesis of the thyroid gland and congenital toxoplasmosis with severe inflammatory changes in the hypothalamus and hypophysis in an infant aged 3 months with clinically pronounced myxoedema was recorded. On this basis were mentioned more recent conceptions of the role of the relation hypophysis-thyroid and hypothalamus for thyroid function. A short account was given of more recent diagnostic methods for the recognition of hypothyroidism and the differentiation between primary (thyroid-conditioned) and secondary (hypophyseal-conditioned) cases of myxoedema: determination of protein-bound and radioactive iodine in the serum; determination of thyreotropin in the blood; investigations of the mucopolysac-

charides in the skin and mast cells, and electroencephalography. As there is no defi[nite] diagnostic sign which is infallable in all cases of myxoedema in children, the diagno[sis] should be established more on investigations of function than on the physiognom[y].

A case of typical congenital hypothyroidism in a boy, aged 12 years, was record[ed]. The case had previously been diagnosed as Hurler's syndrome on account of cert[ain] superficial points of similarity but X-ray pictures revealed very marked dysgene[sis] of the epiphyses together with characteristic changes in the spinal column. In [this] case, thyroid treatment had a pronounced effect: in the course of 6 months, the ch[ild] trebled the number of his centres of ossification.

Discussion: *E. Gjorup:* Do any of the diagnostic signs mentioned indicate the prognosis or provide guidance as regards the dosage? — *Henning Andersen* drew attention to a schema in Wilkin's "Textbook of Endocrinology" which conveniently and lucidly registers the effect of the treatment. Skin biopsy may possibly render a criterion as regards dosage. — *P. Plum:* Is it still correct to employ up to subtoxic doses therapeutically, and are not the majority of the children concerned treated with too small doses? — *Henning Andersen:* Yes. Contrary to *P. Brøstrup*, we had experienced no unfavourable effects of the largest possible doses. — *J. Flamand-Christensen* was of the opinion that normalization of growth may be achieved by means of subtoxic doses. The development of intelligence is uninfluenced by the large doses. — *A. Rothe-Meyer* emphasized that with regard to the mental condition also, subtoxic doses ought to be maintained. Periodic increase of the dose produces periodic increase of mental development. Subtoxic doses are possibly an experiment, but as yet nothing better is available. — *Sv. Brandt:* There are 2 forms of myxoedema and there is perhaps cerebral agenesis in some cases. These latter are probably less influenceable by the treatment. — *Henning Andersen:* EEG investigations of these children should be performed to a greater extent. Mentioned that lower I.Q.s are frequently found in patients suffering from myxoedema with severe EEG changes.

Proceedings of the Section for Pediatrics and School Hygiene of the Swedish Medical Society

Meeting, November 28, 1953

F. Karlström and N. Tunestam: **Risks of infection in children's wards. Ten years' experience of the incidence of nosocomial infections in a children's department.**

The frequency of cross-infections has decreased during the ten-year period. [In]fection occurred in $^1/_3$ of the cases during the first week of hospitalization and in about $^3/_4$ of the cases during the first three weeks. Mostly it is upper respiratory infection. There is less during summer and autumn. The younger the child the more is he exposed to nosocomial infections. Children kept in small units are much more free from infection than children in larger units. Three children died from nosocomial infection and in 12 cases this infection contributed to the death. The increased costs due to the prolonged stay in hospital from nosocomial infection were about 300000 crowns during the whole ten-year period.

Discussion: *G. Herlitz:* Is it clear that the prolonged time of hospitalization is the result of nosocomial infection or are nosocomial infections caused by a long stay in hospital? — *R. Bergman:* Why has not the big problem of epidemic diarrhoea been mentioned? — *B. Söderling:* Many children fell ill after their return home following discharge from the hospital. These children are not accounted for in the reports. — *F. Karlström:* Children who stay a longer time in hospital are, of course, more strongly exposed to infections. It is, however, difficult to assess what role this circumstance plays. The deductions about economics can certainly be criticised but in general they appear to be true. We did not pay attention to the cases from the big epidemic of diarrhoea because we believed they should have troubled the ordinary conditions.

B. Söderling: **Studies of the problem of mother and child in hospitals.**

The studies concern the psychic damages that may be produced by short hospitalization periods for acute diseases or for examination. These disorders should not be regarded as unimportant. Sick children in the sensitive age-group should as much as possible be treated at home or, if hospitalization is necessary, they should keep contact with someone of the family—mother, father, nurse etc. Measures of a painful or frightening nature should be avoided as much as possible. The personnel often lack understanding of the importance of being a mother substitute for the hospitalized child. A child recently admitted has more need of a play therapeutist to give him sufficient time to adjust himself to the new surroundings. One should not try to judge the mother's efficiency on account of her acute mental stress or superficial attitude at the time of admission. Changes in the manner in which the child behaves and in its emotions occurring during hospitalization are important and should be studied. The mother's own problems in relation to the child's mental health should be considered. We should give more time to talk to visiting mothers.

Discussion: *S. Ahnsjö:* On child psychiatry wards the visits of the mothers are unlimited. On ordinary wards the nurses often take a negative attitude to the question of free visiting. A sufficient number of personnel is very important in order to permit plenty of time for talking with the children and to tell them in advance what they have to undergo, for instance before a roentgen examination. — *P. Selander:* During the last year this problem has been studied on 2000 children that have been admitted to Flensburg's Hospital, and in only one case the mother has complained of the interruption of contact with the child. S. was not quite sure whether it really was an advantage to the children to have visits from the family members. — *Y. Åhrén* has started with more liberal visiting hours at the Children's Hospital in Gothenburg. The mother is allowed to visit her child during its meal times if she wants and has the time. It is important that the child be prepared for the interruption of the contact with its mother and also before the different procedures that have to be carried out. The indications for admission to hospital should be very strict. — *K. Gunnarsson:* As a child psychiatrist at an out-patient department one gets quite another view of the incidence of mental damage from hospitalization than to doctors at the hospitals. It is very often exactly those children that have been good and cooperative in hospital that have been most traumatisized. — *B. Nordenfeldt:* About 1 per cent of children in the sensitive age-groups that are admitted to hospitals get some form of mental damage and it is not known how rapidly this disappears. Also iatrogenic measures can sometimes hurt the child mentally. — *A. Wallgren:* In the

absence of Dr. Nordlund I wish to say a few words about our experience. In our clinic at the Karolinska Sjukhuset every-day visits were introduced in 1951. The more experience we have got the more evident it has become to us that these daily visits are valuable for the children. It is important that everything is done to prevent mental damage of the hospitalized children, even if the frequency of this damage is perhaps exaggerated. The problem is international and has been taken up, among many others, by WHO. Next autumn a discussion in Stockholm is planned between paediatricians and child psychiatrists about the problem of how to take care of children in hospital. — *B. Vahlquist:* Hospitals with patients from the country have more difficulty in arranging daily visits from the mothers and keeping the contact between mother and child. An important factor in the mental care of such children is education of nursing personnel in these problems. — *P. Fritsch* reported experiences of his own two children when they were admitted to hospital, one for an eczema, the other for diarrhoea. It is desirable that there be a better contact between parents and children during hospitalization if such a contact is possible. — *E. Fritzell:* In England children usually are treated at home ambulatorily and only extreme cases hospitalized and separated from the mother. — *F. Karlström:* If the mothers are permitted to make very frequent visits on the wards to call upon their children an increased risk of infection is produced. Against this disadvantage must be weighed the advantage that children are emotionally and mentally better off in the presence of their mothers. This latter factor may very well outweigh the increased infection risk. — *S. I. Rollof:* In ambulatory practice it is important that the child has satisfactory confidence in the person who accompanies him, giving him a sense of safety. This may be the father, another family member, and it is not necessary that it should be the mother, because some mothers are very nervous and produce a bad influence on the child that makes the examination difficult. — *B. Söderling:* The problems of the personnel are very important. The children must get information what is going to happen. One detail that should be mentioned is that the children should not be left alone the first evening in hospital.

B. Kihlblom: **Day nurseries and family day nurseries. A psychological study of the advantages and disadvantages.**

The series comprises 60 children 4–6 years of age, half of them from ordinary day-nurseries and the other half consisting of children who during the day are taken care of in private families, controlled by the Children's Welfare Board in Stockholm, so-called family day nurseries. All were only children and had belonged to the nursery for at least 4 months. Keeping the children in groups in ordinary day nurseries seems to be of some importance for a healthy development as it promotes greater independence. On the other hand, the day nursery children are often more frightened and overexcited in the evenings and more often infected. An important advantage with the day nurseries is that they can offer better continuity of care. The children do not suffer from frequent changes as the family day nursery children often do. The family day nursery children live more home-like in small siblings groups. The daily routine is more smoothly adjusted to the individual need of the child. The day nursery is said to be a more neutral surrounding from an emotional point of view. There were no proofs that the family day nursery care was more individual and there was no discord between the two home surroundings.

Discussion: *Y. Åkerrén:* The experiences from Gothenburg are the same as those from Stockholm. The family day nurseries have been in use some years and have

generally been very much approved by the community. — *S. Ahnsjö:* We have got another support for the thesis of the value of the family and the home in comparison with the collective care of children. The result of this study must not be interpreted as a proof that the family day nurseries are more advantageous than ordinary day nurseries. — *P. Selander:* The experiences from different cities are very different as to the advantages of the family day nurseries. The incidence of infections among the children in a collective day nursery shows the importance of introducing the system with family day nurseries. Often the personnel of the day nurseries do not approve of the family day nursery system. — *P. Nordenfelt:* One should be chary of enthusiasm. One should not exaggerate the value of family day nurseries. Another problem is that if family day nurseries are going to be more common it will be difficult to get ordinary foster homes, because the family day nursery care of the children is better paid than foster home care. — *B. Kihlblom:* The investigation is to be regarded as a preliminary one. The groups are small and one should be careful in evaluating the result.

S. Kraepelien: **The incidence of asthma among Swedish school children.**

The incidence in primary public schools during the last two years was 0.73 per cent and the number of children studied was 235000 and 247000 respectively. In the high schools (number of pupils 87000–128000) the incidence during the last eight years varied between 0.38 and 0.58 per cent. These figures must be regarded as minimum values. A special study of the conditions in the public schools in Stockholm in the spring of 1953 (60000 pupils) showed an incidence of 1.37 per cent.

E. Bengtsson: **Working capacity of healthy children, studied with bicycle-ergometry.**

The pulse rate increases in a linear fashion with the working intensity in different age groups, up to a pulse rate of 170 per minute. Then this relationship dissociates, varying with different age groups. The working capacity maximum is attained at a pulse rate of about 200. The pulse-working relation up to 170 signifies the rapidity with which the circulation adjusts itself by increasing the heart frequency to approach its maximum and thereby also its maximum for sufficient circulation. There is no sex difference. Fat children had, in relation to age and weight, a more rapid increase of the heart beat than children in general. It seems to be possible to study the functional capacity of the heart, for instance in malformations, myocarditis and valvular diseases, by pulse recording during bicycle-ergometric examination according to Sjöstrand.

Birgit Jonsson: **The survival time of erythrocytes in morbus caeruleus.**

Determination of the survival time of erythrocytes according to the method of Ashby shows that erythrocytes from patients with low arterial oxygen saturation in cardiac malformations have decreased survival time (60–80 days) if the blood is kept in an anoxic milieu or if it is transfused to individuals with normal arterial oxygen tension. Normal erythrocytes have a normal survival time even in patients with low arterial oxygen tension. The cause of the decreased survival time must therefore be that abnormal erythrocytes are produced during the anoxic state. In connection with anastomosis operations according to Blalock there is a rapid decrease in the survival time during the first days following operation, but then the slope of elimination is normal. The decrease is not greater than may be explained by loss of blood.

Discussion: *B. Vahlquist:* What is the survival time of the erythrocytes three six months after a successful operation? The erythrocytes are macrocytic in newborn in the cyanotic child they are normal or microcytic. — *B. Jonsson:* So far no study has been made three and six months after operation. The erythrocyte diameter in these patients is in some cases small but usually normal. The osmotic resistance is also normal.

R. Lundström: **On the treatment of purulent pericarditis.**

Report of a case of purulent pericarditis due to haemophilus influenzae type B in a six year old girl, treated by puncture of the pericardium combined with local and parenteral treatment with penicillin and streptomycin and introduction into the pericardial cavity of streptokinase and streptodornase (Varidase). In the same manner a concomitant bilateral empyema was treated. The child was discharged well.

Discussion: *A. Wallgren:* Very often penicillin is of no use against haemophilus influenzae because of resistance. Could not the air in the pericardial cavity after puncture be of advantage in preventing concretio? — *R. Lundström:* The bacteria, which were isolated in the first test only, were highly sensitive to all antibiotics, especially penicillin and streptomycin. Experience in pneumothorax treatment does not seem to speak in favour of a prevention of synechiae by the air.

R. Berfenstam, T. Edlund and L. Zettergren: **Some views on the pathogenesis of hyaline membranes.**

Young rabbits were kept for two days in 70–80 per cent oxygen. The ciliary function, studied by the ability of the tracheal mucous membrane to transport siliconized carbon, was studied. It was shown that more than half of the animals did not show any ciliary movements. This was not due to an increased amount of mucus. Hyaline lung membranes have been observed in most of the oxygen-exposed animals in this study, when the animals had been kept for 4–5 days in 70–80 per cent oxygen. Characteristically, those parts of the lungs which have membranes are not atelectatic. The essential pathogenesis of the membranes is probably a capillary damage disorder with exudation of the substance (glycoprotein) from which they are produced. Another important factor is probably the respiratory air that models the exudate.

Discussion: *B. Hellström:* Newborn mice, during exposure to 100 per cent oxygen, develop serious pulmonary damage after a few days, especially in the form of a lesion of the alveolar epithelium and the alveolar capillaries. In some cases acidophil masses were seen in the alveoli and bronchioles. The histological picture did not correspond to the picture of hyaline membranes in premature infants. Above all, the characteristic atelectases were absent. — *V. Åkerrén:* Has the importance of humidity been studied? — *R. Berfenstam:* Dry as well as humid oxygen has been used without any difference in the frequency of the membrane production. The aerosol mist treatment of hyaline membranes in newborns is of some theoretical interest. The viscous, albumen-rich substance that produces the membranes is diluted and the tendency to membrane production is therefore less.

C. Berglund and R. Zetterström: **Malignant collagenosis.**

Report of 6 cases personally studied.

(To be published in Acta Pædiatrica.)

Discussion: *V. Oldfeldt* reported a probable case of collagenosis in a six year old girl that since infancy had had relapsing fever and erythema nodosum-like exanthem, anaemia with granulocytopenia and mild splenomegaly. Improved by ACTH and cortisone. — *H. Enell:* In his department a girl was treated during the last two months for Libman-Sacks' syndrome. She has been ill for one year with symptoms and signs similar to those in the cases of malignant collagenosis described. No L.e. cells were found. Electrophoresis showed increased γ-globulins, 36.3 per cent. No eosinophils in the blood. She shows increasing impairment, although she has been treated with cortisone and ACTH.

J. Winberg: **Metabolic studies during the treatment of so-called D-vitamin resistant rickets.**

(To be published in Acta Pædiatrica.)

Bengt Hagberg: **Case of male pseudohermaphroditism and giant growth in an infant.**

Birth weight 4110 g. Examined at 7 months of age. Body-weight at that time 14 kg and height 75 cm. External genitals were of feminine type. The labiae minora small, no clitoris. Introitus vagina at normal site. Vagina was in the normal position, 4 cm long. Prostate-like mass was felt by rectal palpation but no internal female genitals found. Exploratory laparotomy disclosed that the Pouch of Douglas corresponded to that in boys. On the back of the bladder a ridge was found which was regarded as being the rest of internal female genitals. Biopsy of the sexual glands disclosed on the right side an embryonic testis-like structure without evident interstitial cells. Adrenal function normal. Urine content of oestrogens less than 12.5 M.U. per day. Sella turcica normal. Skeletal development normal. At 23 months of age the child is still large, body-weight 17.5 kg, height 89 cm. The hormonal titers are normal.

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